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THE UNITED STATES ARMY MEDICAL DEPARTMENT

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Perspectives

Commander's Introduction

MG Philip Volpe

Among the many important responsibilities I have assumed as Commanding General of the US Army Medical Department Center and School (AMEDDC&S) is publication of military medicine's very own professional periodical, the *AMEDD Journal*. I am pleased to present the first issue of the *Journal* to be published during my tour. The *AMEDD Journal* is the only periodical published by military medicine for medical professionals to present and discuss topics which stimulate, enhance, and advance the science of military medicine, the primary mission of which is to ensure the health and combat effectiveness of our nation's military force. The scope and application of such professional writing is, of course, not limited to the practice of medicine in the military environment. The majority of the information found in the pages of the *AMEDD Journal* is relevant, and often directly applicable, to the civilian world of medical science and healthcare delivery. Throughout the history of military medicine are found examples of discoveries, advancements, and developments that found immediate application in civilian healthcare, thereby benefitting human society as a whole.

In 1994, The Surgeon General directed the AMEDDC&S to establish an Army Medical Department periodical to be a forum for military medical professionals to present and discuss current healthcare topics and issues, as well as combat theater experiences, for the advancement of military medical science and doctrine development. Since that first issue in October of 1994, the *AMEDD Journal* has evolved in scope, sophistication, and presentation of its content. The *Journal* joined the ranks of the world's most respected medical periodicals in 2009 when it was chosen by the National Library of Medicine for inclusion and indexing in MEDLINE, the nation's premier bibliographic database of life sciences and biomedical information. Indicative of that recognition is the broad readership reflected in its distribution. Not only is it welcomed by Army, Navy, Air Force, and Defense Department recipients, the *Journal* is also found in many civilian medical schools, libraries, and research institutions, as well as foreign military medical organizations and commands. The *AMEDD Journal* is a superb presentation of who we are and what we do as healthcare professionals, both on the battlefield and in garrison.

The *AMEDD Journal* is an invaluable clearinghouse for the most important healthcare information and combat experiences related to maintaining a healthy, viable, effective fighting force. The *Journal* receives manuscripts from across the entire spectrum of medical professionals, including highly experienced medical and dental practitioners, accomplished research scientists, preventive medicine and public health specialists, veterinarians, healthcare support and service specialists, and nonmedical professionals who contribute to healthcare delivery in various ways. The diversity of subject matter is clearly indicative of the depth and breadth of responsibilities and functions found in today's military medicine.

This issue was sponsored by COL Mustapha Debboun, the senior medical and veterinary entomologist at the AMEDDC&S and Chairman of the *AMEDD Journal* Editorial Review Board. For the seventh consecutive year he has organized and assembled an outstanding collection of articles featuring topics related to public health, preventive medicine, and force health protection. Time and again throughout military history, the success or failure of battles, campaigns, and even wars has been determined by the health, and therefore the effectiveness, of a fighting force. There are many examples which demonstrate that it does not matter if a military force has the best training, the finest equipment, and the most capable leadership if the Warriors themselves cannot physically perform their tasks. Indeed, our primary mission at the Army Medical Department begins and ends with the health of the individual Soldier, from his or her first day at basic training until the last day in the Army. Force health protection is the keystone to ensuring that a commander has enough healthy, effective Warriors where and when needed to initiate, sustain, and complete all operations dictated by the unit's mission.

EDITOR'S PERSPECTIVE

One disease threat that has plagued humankind throughout history is rabies, and it remains present in one form or another in almost every area in the world. Even though the well-understood threat of rabies is the subject of repeated training throughout military units, and regulations are established to minimize the potential for contact with infected animals, it still occurs. Indeed, in 2011 a US Army Soldier died from rabies he acquired from a dog bite in Afghanistan. This occurrence, albeit rare, serves to underscore the inescapable facts that rabies exists, and it still kills humans who become infected if prompt notification and treatment do not follow. This threat is even more serious to our military personnel who find themselves in areas where rabies may be endemic, not an uncommon situation. Edwin Cooper and COL Debboun open this issue of the *AMEDD Journal* with an important article that reinforces

the importance of understanding that the threat of rabies to military personnel exists, it is serious, and what is necessary to minimize the potential of contracting this pernicious disease. This article should serve as a refresher in what to do and, perhaps more importantly, what not to do in combating the horror that is rabies.

The value of the military working dog (MWD) in many environments, including garrison security, disaster recovery, and combat operations, is now an indisputable fact. Each of those dogs represents a significant monetary investment in procurement, training, and support. Further, as clearly demonstrated throughout the last issue of the *AMEDD Journal*, specially trained dogs are increasingly found in medical treatment roles within the military, whether for physical assistance, in psychological and physical therapy settings, or as companions for emotional support. Understandably, the health of these dogs is important, not only because of the monetary investment, but also because dogs can carry diseases transmittable to humans. In their article, CPT Lee McPhatter and his coauthors report on their investigation of the insect vector of one such disease on military installations in and around San Antonio, Texas. As pointed out in their article, not only is Chagas disease a significant health threat to MWDs, but it also sickens over 7 million people worldwide, and kills approximately 21,000 each year. Their important article is a detailed presentation of a well-designed, carefully executed, thorough search for triatomine bugs, not only in areas where they represent a threat to dogs being raised and trained for service in government agencies, but also in military training areas where the potential to infect humans is very real.

Leptospirosis is another zoonotic disease that presents a significant threat to humans worldwide, especially in areas where military deployments may be expected, such as combat environments and scenes of natural disasters. Therefore, it is very important that medical caregivers have the capability to detect the *Leptospira* bacteria in austere environments, where infrastructure and transportation resources may be limited at best. James McAvin and his coauthors present their work in the development of a field-expedient method to identify the presence of *Leptospira* in rats, the most significant reservoir of the disease causing agents. Their article is an excellent example of a carefully designed and meticulously executed research project, presenting the methodology and science of their research with clarity and detail. The results of their efforts should provide caregivers with the capability to detect another serious disease threat in remote, austere locations, allowing them to quickly narrow the field of diagnostic possibilities in the treatment of sick personnel.

The capabilities of our fighting forces become increasingly sophisticated and effective as their training, equipment, and support infrastructure continue to evolve in response to the ever-changing nature of the enemy threat. Detection, identification, and analysis of that threat is essential to formulating the appropriate response. The same principle applies to the threats they face from vector-borne diseases which can vary significantly across geographic locations and climates. In their detailed article, MAJ Michelle Colacicco-Mayhugh and her coauthors describe the current structure and functioning of the US military's entomological support in the Afghanistan theater of operations. This article provides excellent insight to the complex coordination and planning that is necessary to ensure that entomological resources are available when and where needed in the challenging, widely dispersed, fluid, and dynamic combat environment of Afghanistan.

Organophosphates and closely related chemicals are toxic substances routinely introduced into our environment because they are found in a number of commercially available insecticides. However, the same substances are components of potent neurotoxic chemical warfare agents, such as sarin. The potential risk posed by either application has, of course, stimulated a number of approaches to decontamination, from simple bleach to specifically developed decontamination compounds. But most techniques to determine the effectiveness of decontamination efforts involve expensive equipment and trained technicians. Retired CDR David Clayborn and his colleagues from Missouri State University investigated the effectiveness of a relatively simple and inexpensive bioassay technique using measured survival of red flour beetles to judge the level of toxic residue. The article is the report of their detailed, rigorous research project which evaluated the effectiveness of that approach. The results of the study are encouraging, demonstrating that the approach offers the potential of a rapid, very low expense bioassay that accurately measures biological toxicity of surfaces previously contaminated with malathion and malaoxin. This technique may be developed into an initial screening tool for field environment applications to determine the decontamination of a variety of toxic environments.

In a 2010 *AMEDD Journal* article, Dr Coleen Baird discussed the emerging recognition of the potential hazard to the respiratory health of deployed personnel (and others) posed by open burn pits around bases in Iraq and Afghanistan. In 2011, she followed with another article investigating the questions of increased incidences of respiratory difficulties among troops returning from combat deployments. The increasing interest and concern about these topics led to a Department of Veterans Affairs request that the Institute of Medicine form a committee to determine the long-term health effects from exposure to burn pits. In her latest article, Dr Baird examines the resulting report, describing the approach used by the study committee, and their findings. She also discusses the responses to the report by the military and the VA in their continuing efforts

to definitively understand, and potentially quantify, any relationships between burn pit exposure and the health of deployed personnel. Dr Baird's excellent series of articles continue to highlight a subtle, enigmatic, but potentially serious threat to the health of our Warriors, both while deployed and after return.

In a complementary article, Jessica Sharkey examines the broad topic of all types of potential inhalational exposures to US military forces throughout the 10 years of Southwest Asia operations using records of in-theater medical treatments, medical evacuations, and postevacuation care. Her well-researched article develops the scope and character of inhalational threats, explains the difficulties involved in categorizing and quantifying exposures, and presents the available data related to such exposures throughout the decade of deployments. Ms Sharkey's article provides more insight into the difficulty that medical science faces in its efforts to address health issues resulting from the broad range of potentially harmful inhalational contaminants.

Posttraumatic stress disorder (PTSD) has been recognized, under various descriptive names, as a diagnosis mode by medical professionals for more than a century. Mental and behavioral health specialists have long been involved with research and studies as to its causes, diagnosis, and treatment, seeking to understand how and why it affects individuals to such varying degrees. In their article, LTC Sandra Escolas and her coauthors describe their work exploring how an aspect of an individual's ability to relate to others, known as attachment theory, may be a factor in how that individual deals with the stresses that induce PTSD, and influence his or her ability to cope with and recover from its symptoms. This is a carefully designed, extensively researched, meticulously conducted scientific study that produced statistically sound results. The insights contained in this important article should be of value to researchers and practitioners involved in the care and treatment of those diagnosed with PTSD.

Military personnel must maintain a high level of physical fitness throughout their military careers, and running is an integral component of the fitness regimen. Unfortunately, running all too often results in a number of injuries to the feet and legs, some of which are serious and debilitating. Over the last several years, various alternative running styles have been developed and promoted among running enthusiasts, most intended to change the footstrike to one believed less prone to cause injury. LTC Donald Goss and Dr Michael Gross conducted an extensive search and review of published information and data on the various styles to identify any biomechanical advantages, injury relationships, and trends associated with a specific style. Their article is a careful compilation of data from numerous, diverse sources, and a detailed presentation of their analysis which should be of great interest to all practitioners involved with lower body injury prevention and rehabilitation.

LTC Scott Shaffer and his colleagues return the Army-Baylor University Doctoral Program in Physical Therapy to the pages of the *AMEDD Journal* with a report on their study investigating the presence of neuropathy in the wrists and hands of Army dental assistants at the beginning of their training. Since dental personnel are reported to have a relatively high prevalence of upper-extremity musculoskeletal disorders, this study sought to identify preexisting conditions which might be exacerbated by the movements and positions required for their work. Indeed, they found that fully 11% of the sample population had indications of abnormalities that could be predictive of future disorders as they pursue their occupations in dental treatment. This well-designed and executed research project is another example of level of professional expertise that military medicine directs towards providing only the best "care for the caregivers" across all of our specialties.

Radiation is a word that reflexively strikes fear in most people, primarily due to the overall lack of knowledge about it, combined with the exaggerated and/or false presentations of radiation exposure in popular media. The problem of preventing inaccurate information and distorted claims from causing undue anxiety and even panic is exacerbated by today's instant, pervasive, and completely unregulated worldwide communications. COL Mark Melanson and his coauthors use 3 actual events of potential radiation exposure within the US Army to develop their article on the very important topic of communication of radiation risks. In this interesting and very informative article, they clearly explain the complexities inherent in the subject of radiation risk, the common misconceptions that must be addressed and countered, and the compounding difficulties presented by addressing it within the military context. The principles of risk communication discussed in this article are important and widely applicable for all medical professionals in this dangerous era of weapons of mass destruction.

In July 2011, the Armed Forces Health Surveillance Center, the Center for Disaster and Humanitarian Assistance Medicine, and the US Central Command sponsored a major regional conference in Abu Dhabi to promote collaboration and interoperability in responding to complex health and humanitarian emergencies. In their thorough and very informative article, Priya Baliga and her coauthors describe the diversity of attendees, the genuine interest and enthusiasm demonstrated by the participants, and the diversity of the valuable information shared throughout the conference. The reader can only be encouraged at the apparent tone of cooperation and mutual respect demonstrated throughout, as health professionals meet and interact as colleagues rather than competitors in the common goal of healthier populations throughout the region.

The Relevance of Rabies to Today's Military

Edwin D. Cooper, BS, MT
COL Mustapha Debboun, MS, USA

A 24-year-old Army Specialist was assigned as a cook at Combat Base Chamkani, Paktia Province, Afghanistan, from May 2010 to May 2011.[1] He was a known animal enthusiast and had been caring for unauthorized dogs in his unit's area, in spite of General Order Number 1 which forbade the keeping of animals in theater. Feral dogs have been a perennial issue throughout the combat theaters of Iraq and Afghanistan, as they are attracted to the presence of food waste at dump sites around the bases.[2] During an attempt to break up a dog fight between one of the local unauthorized dogs and a feral dog, the Specialist was bitten on the hand. According to reports, the Specialist told his mother that he was ordered to shoot the feral dog and have it sent for rabies testing. It has also been reported that a rabies vaccine series was initiated, but discontinued because the vaccine had expired. Unfortunately, there is no evidence that any animal was tested for rabies and no record of medical treatment being sought or given. The Specialist left Afghanistan in May 2011 with his unit for Germany. He later reported to his new assignment at Fort Drum, New York, in August 2011. By this time, he had started to exhibit neurologic symptoms. He complained of a tingling pain radiating down his arm on August 14, and was treated for tendonitis at a civilian hospital. He reportedly had trouble drinking on August 17 and collapsed at work on August 19, again seeking treatment at a civilian hospital. At this point, it was learned that he had received the dog bite in Afghanistan. This history and the symptoms led to the suspicion of rabies, which triggered specific testing at Wadsworth Center, the New York State Department of Health's public health laboratory. Rabies virus antigens were detected in hair follicles of nuchal skin biopsy specimens by direct immunofluorescence, and rabies viral RNA was found in saliva and cerebrospinal fluid (CSF) by reverse transcriptase–polymerase chain reaction. Once rabies was identified as a potential cause of his symptoms, a coma was medically induced as part of an experimental rabies treatment protocol. At the time of his hospitalization, rabies virus specific immunoglobulin M and immunoglobulin G antibodies were detected in his serum and cerebral spinal fluid in laboratory testing performed by the Wadsworth Center and at the Centers for Disease Control and Prevention (CDC).[3] Virus neutralizing antibodies were found in the serum at a level of 0.07 IU/mL on August 28, and by the date of his death on August 31, had increased to 0.50 IU/mL. Virus neutralizing antibody was not detected in the CSF. Laboratory testing at the CDC detected no rabies virus neutralizing antibody in the blood drawn in May 2011 as a routine banked sample upon his return from deployment. He suffered a massive brain hemorrhage and succumbed to rabies on August 31. He was the first US military member to die from rabies since the Vietnam war.[1] Postmorten tests performed at the CDC established that the virus variant was consistent with the canine rabies virus found in Afghanistan.[3]

The Army and local health departments, in collaboration with the New York State Department of Health and the CDC, interviewed and provided risk assessments to the patient's family members and friends, fellow travelers, health care workers, and any other personnel who may have interacted with the patient.[3] Any individual that was identified as meeting the exposure criteria set by the Advisory Committee on Immunization Practices was given postexposure prophylaxis. The exposure criteria includes wound or mucous membrane exposure to the patient's saliva, CSF, neural tissue, or tears.[4] The Army Public Health Command assembled a rabies response team to locate other members of the Soldier's unit who may not have reported bites to medical providers while in theater. More individuals were identified through medical record reviews. Any Soldier who had a possible animal exposure was evaluated and, where indicated, given postexposure prophylaxis, which typically consists of immediate administration of rabies immune globulin, followed by a series of 4 antirabies vaccinations given on days 0, 3, 7, and 14.[5] Postexposure prophylaxis has proven to be very effective when given promptly, however, rabies is considered to be universally fatal once clinical signs develop. In all, about 9,000 personnel were evaluated to assess their risk of rabies exposure.[1]

REPORTED ANIMAL BITES

The Armed Forces Health Surveillance Center (AFHSC) reported 643 animal bites to US personnel in Southwest Asia and the Middle East combat operational theaters from 2001 through 2010, half of those being dog bites.[6] A total of 117 personnel reported that they received some amount of rabies vaccine. Of the 20,522 total animal bites reported from US Armed Forces worldwide during that period, the majority of the reports from outside

the current combat operational theaters were from dog bites.[6]

Rabies has been known since ancient times, with the first basic treatment for rabies demonstrated by Louis Pasteur in 1885. Still, as of 2001, every 15 minutes one person died from rabies and over 300 were exposed.[7] Most exposures to rabies worldwide are due to bites from canines. Worldwide, the rabies virus kills an estimated 55,000 people annually, with about 56% of the deaths in Asia and 44% in Africa.[8] The majority (84%) of these deaths occur in rural areas. In many of these places, there are often great difficulties to overcome in the transport of medical supplies, especially those requiring cold storage. Worldwide, there is a limited supply of rabies immune globulin.[9]

SURVIVORS ARE EXCEPTIONS

Rabies is considered to be the infectious disease with the highest case-fatality ratio.[10] There have been only a handful of recent cases in which a patient has survived after symptoms were exhibited, each having had circumstances that may have weighed in the patient's favor. As described by Willoughby et al,[11] in 2004, a girl aged 15 received a bite on the finger while catching and releasing a bat from inside a building in Wisconsin. The finger was washed and disinfected with hydrogen peroxide. No medical attention was sought at the time and no post exposure prophylaxis was administered. A month later, she felt symptoms including nausea and vomiting and sought medical attention. Her symptoms progressed to include fever, salivation, and difficulty in swallowing. Laboratory tests of serum and cerebral spinal fluid showed the presence of antirabies antibodies. As part of a new and aggressive treatment regime, the patient was placed in an induced coma using ketamine, phenobarbital, and midazolam. Ribavirin and (later) amantadine were added for direct antiviral effect. After about 2 weeks of aggressive therapy, the girl began to show positive effects. She was considered to be clear of transmissible rabies by day 31 of hospitalization and was discharged on day 76. The girl initially had severe neurological impairment which has progressively, albeit slowly, improved with time. It is possible that the therapy in this case was more successful than in other cases due to a possible lower dose of rabies virus, early recognition of the symptoms, and aggressive management. The therapy was based on a hypothesis of protecting the brain from injury while enabling the immune system to mount a response and clear the virus. This treatment regime has since been termed the "Milwaukee Protocol." According to the Children's Hospital of Wisconsin Rabies Registry,[12] 4 of 28 rabies patients treated using the Protocol have survived (as of this writing). There is debate among scientists concerning how many of surviving patients actually responded to the therapy, as well as to the appropriate advice for treatment of future patients.[13]

The rabies virus spreads throughout the body through the cells of the nervous system. It is usually introduced into the tissue through a bite, scratch, or mucous membranes from an inoculum of saliva. The virus may remain in the local tissue for some period of time depending on the depth of the wound, innervation to the area, and other biological factors. Upon reaching nerve cells, the virus spreads very quickly and efficiently through the central nervous system, by fast axonal transport along neuroanatomical pathways.[13] The virus travels in a retrograde direction through the axon toward the neuron cell body. The rabies virus is able to take advantage of the axonal transport machinery of the neuron to travel through the axon at speeds of 3 to 10 mm per hour.[14] Once the virus has passed through the brainstem into the brain, the antirabies immunoglobulin is unable to reach the virus, because it cannot pass through the blood brain barrier. Neurologic symptoms are exhibited once the virus passes into the brain. After reaching the brain, the virus then travels outward to the salivary glands where it is available for transmission to the next victim. The rabies virus has evolved a unique and extremely effective mode of transmission, combining placement of the virus with alteration of mammalian behavior to allow it to be passed on to other victims.

US ARMY EXPERIENCE WITH RABIES

The Army has long recognized the threat of rabies to military operations. The Office of The Surgeon General received reports in 1937 of:

> …5,962 dogs, 225 cats, 18 monkeys, 1 fox, and 1 squirrel that were given single dose antirabic vaccination at 67 Army posts during the calendar year 1937. Quarterly Veterinary Sanitary Reports showed that vaccination is practiced at 71 percent of the reporting stations.[15]

According to the 1937 report, the last reported human case of rabies in the military was in 1926. There were 8 cases and 6 deaths reported from 1906 to 1926.[15]

In 1942, the Eighth Service Command Medical Laboratory was established at Fort Sam Houston, Texas.[16] It included a food analysis branch and a virus laboratory, both of which were supervised and operated by veterinary personnel. The virus laboratory was mainly concerned with the study of troop health aspects of such diseases as typhus, Rocky Mountain spotted fever, lymphogranuloma venereum, lymphocytic choriomeningitis, rabies, ornithosis, equine encephalomyelitis, and St. Louis encephalitis. The various Army veterinary

laboratories across the continental United States eventually combined into the Department of Defense Food Analysis and Diagnostic Laboratory (FADL). In 2011, the Army Veterinary Command was merged into the newly established US Army Public Health Command (USAPHC), and the FADL became part of the USAPHC Region-South. Currently, there are 2 Army laboratories with rabies testing capabilities, one at the USAPHC Region-South in Fort Sam Houston, and the other at the USAPHC Region-Europe in Landstuhl, Germany.

ANIMAL HOSTS

The primary carrier for rabies (genotype 1 of the genus *Lyssavirus*) worldwide is canine. This is especially true in urban centers of the developing world. It should be understood, however, that any mammal can become infected by the rabies virus. There are currently 9 recognized antigenic variants of the rabies virus. Each variant is associated with a specific species of mammal that commonly carries the virus within a certain region. This relationship is complex and the genetic characterization of the variants is being redefined as the science continues to advance. Additionally, the habitats of the host species are constantly changing. For example, raccoon rabies in the states of the US eastern seaboard has been slowly moving westward. With wildlife vaccination efforts in Texas, the canine variant of rabies in coyotes has been eliminated in Texas, but persists south of the border in Mexico. Further, it has been reported in Mexico that the bat variant in vampire bats has been found at higher elevations of the mountains due to an increase in average air temperature. Mongoose throughout the Caribbean islands, where they are an invasive species, are now demonstrating a unique challenge in regards to rabies. They have been a particular problem in Puerto Rico, where they not only pose a threat of rabies, but also threaten endangered birds through predation.[17]

In 1942, Fox wrote of the presence of bat rabies in Trinidad.[18] He emphasized the need for inoculation of men and cattle, but also emphasized that rabies must be kept in perspective compared with other communicable diseases found in the tropics. Rabies had been recognized earlier in the century in cattle in Brazil and was linked with vampire bats. Rabies is an increasing economic issue throughout Central and South America due to the impact of virus transmission from vampire bats to cattle and other livestock. It should be noted that bat control efforts may inadvertently have an adverse impact by the decimation of collocated beneficial bat species.[19] Many species of bats cohabit the same roosts as vampire bats. Insectivorous bats are beneficial through the feeding on vast numbers of insects, while many plants depend on the pollinating activities of fruit bats.[20]

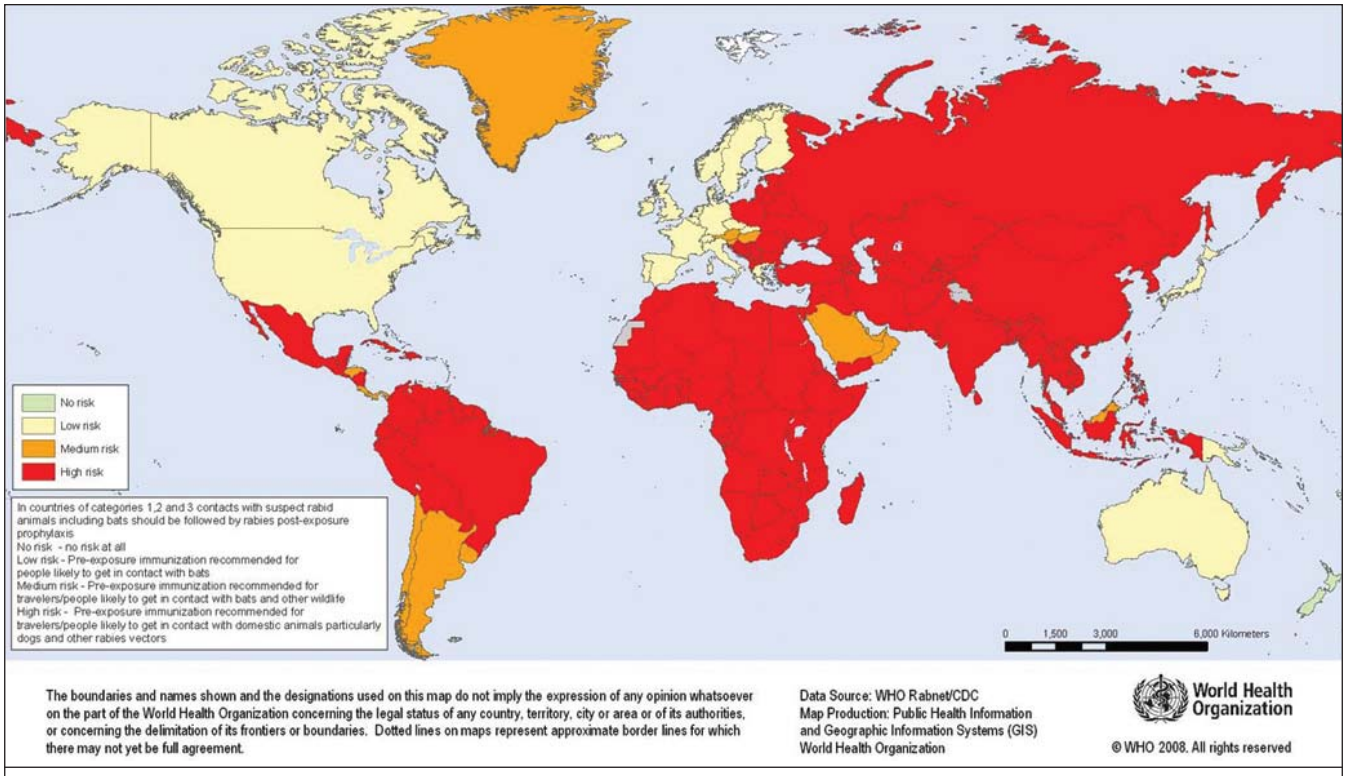
BAT RABIES IN THE UNITED STATES

Bat rabies was recognized for the first time in the United States in 1953. The son of a migrant worker from Mexico was bitten by a bat while searching for a lost ball on a ranch near Tampa, Florida. The bat was knocked to the ground by the boy's mother, and killed by the father. Fortunately, the owner of the ranch knew about rabies in vampire bats in Mexico and insisted that the bat be tested for rabies. It was sent to the local public health laboratory and was confirmed to be positive through the identification of Negri bodies on microscopic examination. Further testing included inoculations of mice which resulted in the 5 injected mice dying with clinical symptoms of rabies. The bat was later identified as a lactating female Florida yellow bat (*Dasypterus floridanus* Miller, 1902). The boy was administered postexposure prophylaxis and, after an initial period of illness, recovered uneventfully.[21]

The following year (1954), a study was undertaken at the US Army Veterinary Laboratory at Fort Sam Houston to investigate the prevalence of rabies in bat species throughout the southwestern United States. A total of 1,247 bats were collected from various sites in Texas, Louisiana, Arkansas, and New Mexico, including 27 vampire bats collected from 2 sites in the northern parts of Mexico for comparison. The bats were necropsied and Sellers stained impressions of brain tissue were examined to look for the presence of Negri bodies. A portion of each brain was also injected intracerebrally into Swiss albino mice to isolate the viral agent. Additionally, serologic studies were conducted using the mouse inoculation procedure. Four species of bats, 3 from Texas and one from Louisiana, were shown to be naturally infected with rabies. This study also demonstrated that a high percentage of the bat brain tissues from which rabies virus was isolated through the use of mouse inoculation had been negative for Negri bodies.[22]

BAT RABIES IN CENTRAL AND SOUTH AMERICA

There has been an increase in the number of human cases of rabies transmitted by bats throughout Latin America.[23] This has come at a time when canine rabies has begun to decline in some areas due to concerted antirabies campaigns. Of the bat cases, the majority are due to vampire bats. It has been surmised that the vampire population has increased over the past several decades due to increasing numbers of cattle operations throughout the region. In areas where the cattle have overgrazed an area and human occupations moved in, the bats simply change their food source from bovine to human, leading to outbreaks of rabies. Other factors involved include the clearing of rain forest cover, poor condition of available housing, and lack of access to health



The levels of the risk of rabies within countries and areas worldwide as of 2008. Source: http://www.who.int/rabies/resources/en/

care. In 2009, a case of rabies encephalitis in a teen aged male in Colombia was considered to be the first case of bat transmitted rabies in an urban area in the region.[24] The virus was found to be the antigenic variant whose main reservoir is the vampire bat, *Desmodus rotundus* (E. Geoffroy, 1810). The Milwaukee Protocol had been attempted, but was not successful due to a number of complicating factors.

RABIES WORLDWIDE

As shown in the Figure, rabies is present worldwide on all continents except Antarctica.[9] Terrestrial rabies, or rabies in mammals other than bats, can be found in over 150 countries. Countries that may be referred to as "rabies free" include Australia, Guam, Iceland, Japan, Norway, Sweden, and the United Kingdom. The World Organisation for Animal Health considers a country to be rabies free for international trade purposes when certain criteria are met.[25] These criteria include having a system for surveillance and notification of rabies, effective importation procedures and other measures. There can be no cases of rabies acquired within the country in animals or man for 2 years and no cases of rabies from imported carnivores outside of a quarantine station. The criteria do not include the isolation of a bat lyssavirus, which can still present as rabies disease in humans. At times a country's claim to be rabies free can be tenuous due to disease reintroduction or emergence. According to the World Health Organization, all of Africa and Asia, Central America, the northern half of South America, and the Caribbean islands are at high risk from rabies. The CDC has estimated the rate of possible exposure to rabies of travelers throughout the world to be in the range of 16 to 200 per 100,000 travelers.[26] Guidelines for rabies treatment to include both pre- and postexposure prophylaxis are specifically intended for use within the United States. These guidelines are currently the best available for deployed environments around the globe, but it must be realized that rabies is a disease which still has many potential unknowns. Rabies virus is the most important of 11 known lyssaviruses globally.[27] The other lyssaviruses cause disease which clinically resembles rabies, however, there is reason to question the effectiveness of pre- and postexposure prophylaxis based on the genetic distance each virus has from the rabies genotype.[28]

RABIES TESTING

The animal suspected of carrying rabies must be euthanized in a humane manner such that the biological integrity of the brain is not compromised. The head should be removed and transported to the laboratory in an insulated box with ice packs. Transportation should be arranged to effect the shipment of the sample to the laboratory as quickly as is feasible. A bat may be submitted as a whole body. The gold standard test for rabies in animals remains the direct fluorescent antibody test

(DFA). This test replaced the use of Sellers stain to determine the presence of Negri bodies in the mid 1960s. In the DFA test, brain tissue is adhered to glass slides in an "impression smear," and, after fixation in acetone, stained with a fluorescent conjugate. The presence of punctate bodies of apple-green fluorescence indicates a positive sample. The limitations of this test in an austere environment are the requirements for a fluorescent microscope and cold storage capability for reagents and for transport of samples.

In 2008, Veterinary Laboratory Europe, now US Army Public Health Command Region – Europe, deployed a veterinary pathologist to Iraq and Afghanistan to provide rabies surveillance capabilities and training to military personnel and key local civilian veterinarians.[29] The US military exchanged ideas and information with their civilian counterparts and shared their common interest in future coordination regarding rabies surveillance in Iraq and Afghanistan. In 2010, a veterinary officer with the 734th Agribusiness Development Team stationed in Kunar Province, Afghanistan, provided continuing education training on rabies and other topics to the private veterinary practitioners in Kunar.[30] This was done as part of an effort to establish a rabies control program for Afghanistan.

VACCINATION PROGRAMS AND SEROLOGY FOR RABIES ANTIBODY

A reccurring theme in public health seems to be the need to reeducate the public on the need for public health programs. The safety and efficacy of vaccines seem to be particularly contentious issues. In 2007, the CDC declared the United States to be free of the canine strain of rabies,[31] indicating that the rabies variant whose natural hosts include dogs and coyotes is no longer considered enzoonotic in the continental United States. This was after many decades of vaccination programs through local and state health agencies across the country. At each stop, the US Public Health Service had to convince local community health decision-makers to allow the program to go forward, often against dissenting voices in the community. A modified version of the Semple phenol killed virus vaccine was recommended by the US Army in the 1940s for use on dogs, but it required semiannual vaccination.[32] Vaccine and vaccination protocols have greatly improved since then. It is important to understand, however, that even though the canine variant may no longer be enzootic, rabies variants from bats, raccoons, and skunks still exist in the United States, and there is always the risk from imported cases of rabies.

In 1988, the canine strain of rabies was spreading in the coyote population in Starr county, Texas.[33,34] The epizootic was spreading through 11 other counties in south Texas, approaching San Antonio. A young boy died from rabies transmitted by a puppy, which had received the virus from a coyote. A new program was initiated by the Texas Department of Health (TDH) to vaccinate the coyote and later the fox populations through the use of oral bait.[35] Since 1995, the FADL Diagnostic section has evaluated coyote and fox serum by Rabies Fluorescent Focus Inhibition Test (RFFIT) as a means of monitoring the efficiency of vaccine baiting strategies in the field. The use of titer information has allowed the TDH to adjust flight plans, distribution rates, and other variables to increase effectiveness and efficiency. Each January, bait is dropped over an area of south Texas. In March or April, a cohort of animals is harvested and bled. Samples of serum are sent to the FADL for RFFIT, while other samples are retained by the TDH or sent on to the US Department of Agriculture or the CDC for related research. The program has greatly reduced the risk of transmission of rabies from coyotes and fox to domestic animals and humans.[36]

The RFFIT assay is also used by FADL to evaluate the titer of veterinarians, animal handlers, and other personnel with potential rabies exposures. This helps ensure the safety of having adequate titers of antirabies antibody, while reducing the risk of vaccine-related events by reducing the frequency of vaccination. A side benefit is the inherent cost savings. The CDC Advisory Committee on Immunization Practices recommends that personnel with animal exposure have serum antibody titers tested every 2 years.[4] Those working directly with the rabies virus should be tested every 6 months. The FADL laboratory is accredited by the American Association for Laboratory Accreditation (A2LA) to the ISO 17025 standard, and by COLA laboratory accreditation (http://www.cola.org) for human antirabies antibody testing by the RFFIT method. The FADL laboratory tested 9,620 samples by RFFIT assay from January 2000 through December 2011.

Animal travel has become a major concern for the Department of Defense. Since 1997, the FADL has offered the Fluorescent Antibody Virus Neutralization (FAVN) assay for rabies antibody testing for military working dogs as well as pets of service members going overseas. The FADL is one of only 2 laboratories in the United States that offer this test, the other being at Kansas State University. This testing has allowed the movement of animals to Hawaii, Britain, Europe, Japan, Guam, and other countries without the stress of prolonged quarantine. The laboratory is accredited by A2LA and the French Agency for Food, Environmental, and Occupational Health and Safety, the accreditation laboratory in Nancy,

France, which authorizes the results for international travel to Europe. The FADL laboratory tested 88,545 samples by the FAVN test from 1999 through 2011.

FERAL ANIMALS SHIPPED FROM IRAQ

When US forces entered Afghanistan in 2001, General Order Number 1 prohibited military members from any contact with stray dogs. Over time, some military personnel began to ignore the order and "adopt" strays as pets. In June 2008, an international animal rescue group imported a shipment of 26 animals from Iraq to Newark, New Jersey.[37] The 24 dogs and 2 cats were to be reunited with those personnel who had befriended them in theater. On arrival, one cat was found to be ill with neurologic signs. The cat was euthanized and tested negative for rabies. Three days later, an 11-month-old dog became ill. It was taken to a veterinarian and hospitalized with fever, diarrhea, wobbly gait, agitation, and crying. The dog's condition proceeded to deteriorate until it was euthanized on June 11. Testing at the New Jersey Public Health and Environmental Laboratory proved positive for rabies. Further testing at the CDC typed the virus as a variant associated with dogs in the Middle East. The dog had reportedly lived with a Soldier in Baghdad for 7 months and was not vaccinated for rabies before transport. None of the 24 dogs had the required valid rabies vaccination certificates. Five of the dogs had a previous rabies vaccination, the remaining 21 animals received their primary rabies vaccine upon embarking transport. By the time the positive rabies results were reported, the 23 dogs and one cat were spread out to California, Colorado, Connecticut, Iowa, Kentucky, Maryland, Massachusetts, Missouri, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Texas, Virginia, and Washington. State health departments in all 16 states were notified, with the recommendation that the animals receive immediate vaccine and be quarantined for 6 months. Additionally, postexposure prophylaxis was recommended for 13 individuals who were identified as having had potential exposure to infectious saliva.[37]

RABIES RISK AWARENESS

Murray and Horvath[38] outlined 6 components of deployment medical preparation the US military uses with the goal of preventing infectious diseases: preparation, education, personal protective measures, vaccines, chemoprophylaxis, and surveillance. While each component is critically important, education of rabies awareness is one in which personnel at all levels of responsibility can assist. The salient features of this approach are to stress the reasons to not pet or feed animals, especially dogs; avoid direct contact with animals and animal products; and not adopt stray animals as pets. In recognition of human nature and the individual's desire to seek the human-animal bond, all personnel must be made fully aware of the risks involved in the choices they make. It is the responsibility of military public health professionals to educate service members at all levels about the risks to health and safety in deployed environments. Although all military personnel deploying overseas receive a predeployment medical threat brief which includes rabies risk information, additional direct emphasis stressing the significance of rabies disease in wild animal populations should convince young Soldiers that their personal decisions with regard to local animals could be a matter of life or death.

The Global Alliance for Rabies Control (GARC) is a partnership of many international agencies with an interest in rabies, including the World Health Organization and the World Organization for Animal Health. The GARC has designated each September 28 as "World Rabies Day." This initiative brings together many different entities to raise awareness about the impact of human and animal rabies, prevention strategies, and efforts toward elimination of this disease from its main sources. The World Rabies Day organization (www.worldrabiesday.org) is a good source of information about educational materials in many languages for a variety of intended audiences. The rabies information page on the US Army Public Health Command website (http://phc.amedd.army.mil/topics/discond/aid/Pages/Rabies.aspx) also has helpful educational materials for rabies risk awareness, including specific information for deployed environments. The availability of such information provides the opportunity for all US military personnel, wherever they may be, to become actively engaged in the prevention of contracting this universally fatal, but entirely preventable disease.

THE CHALLENGE

At the 2012 Military Health System Conference, The Surgeon General of the Army, LTG Patricia D. Horoho, acknowledged the death of the Soldier to rabies[39]:

> Are we good enough when we lose one Soldier to a preventable illness? Last summer, an active duty Soldier died of rabies. Of rabies? This is the first active duty rabies death in over 40 years. So my question to you is… are we good enough? My challenge and my personal belief is that…we can be better! We must be better!

She continued to stress that the Military Health System must find ways to influence behaviors of patients in the "white space" between the 100 minutes a year that an average Soldier has contact with a healthcare provider.

Rabies is an entirely preventable disease if the individual is informed enough to understand the importance of (1)

avoiding contact in the first place, and (2) seeking and receiving treatment if contact occurs. The consequences otherwise are tragic.

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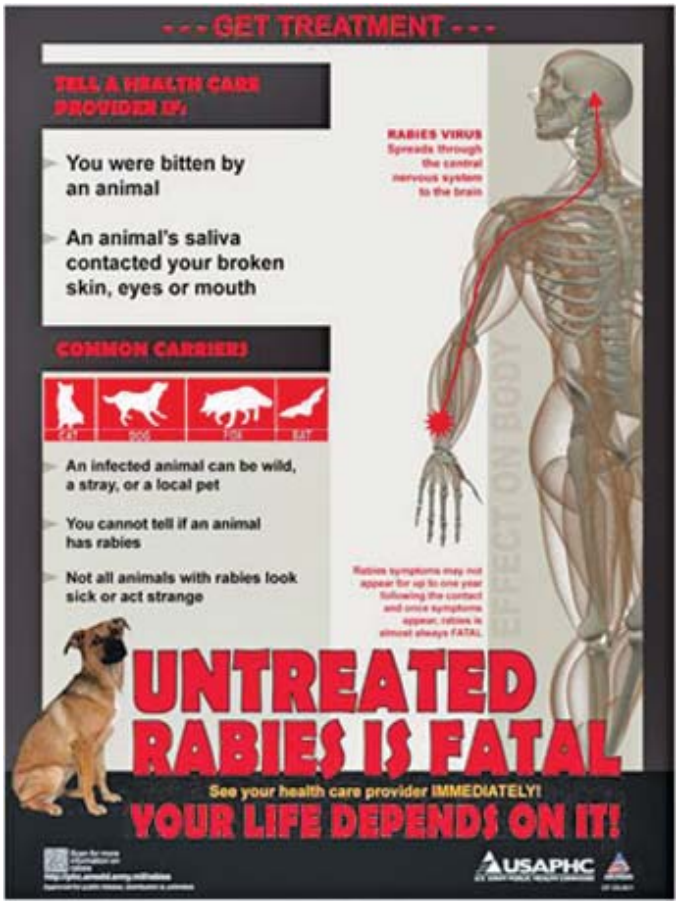

Vector Surveillance to Determine Species Composition and Occurrence of *Trypanosoma cruzi* Infection at Three Military Installations in San Antonio, Texas

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ABSTRACT

Chagas disease, also known as American trypanosomiasis, is caused by the hemoflagellate protozoan *Trypanosoma cruzi* which is transmitted by blood-sucking triatomine bugs (Hemiptera: Reduviidae; Triatominae). The disease is endemic to south Texas, but exists almost exclusively as a zoonosis. Chagas disease has proven to be a serious public health threat to military working dogs. In 2007, seroprevalence of Chagas disease in military working dogs in San Antonio, Texas, reached 8%. A faunal survey was conducted at 3 San Antonio area military installations (Camp Bullis, Fort Sam Houston, and Lackland Air Force Base). A total of 140 triatomines representing 4 species (*Triatoma gerstaeckeri*, *T. sanguisuga*, *T. lectularia*, and *T. indictiva)* were collected. *Trypanosoma cruzi* infected bugs were only collected at Lackland Air Force Base, where the overall infection rate was 16%. The wood excavation technique developed during this study collected all life stages. Only 2 life stages (adult and 5th instar) were positive for *T. cruzi*.

INTRODUCTION

Chagas disease or American trypanosomiasis is caused by the hemoflagellate protozoan *Trypanosoma cruzi* Chagas, which was identified by the Brazilian physician Carlos R. J. Chagas in 1909.[1] It is a serious disease that mainly occurs in Latin America (Mexico, Central and South America).[2] Currently, over 7 million people are infected with more than 108 million people at risk annually.[2] Of those infected, 21,000 are predicted to die each year.[2] *Trypanosoma cruzi* is mainly transmitted to humans via infected feces of hematophagous triatomine (Hemiptera: Reduviidae: Triatominae) bugs. Human transmission primarily occurs when an infected bug defecates on a host during or shortly after taking a bloodmeal. Reacting to an itching sensation, the bite victim rubs fecal material into the bite site, which ultimately facilitates the transmission of the parasite. Triatomine bugs feed on many mammalian and avian hosts. They typically feed on sleeping humans at night, attacking wherever skin is exposed, but especially on the face. For this reason, the triatomine bug is often referred to as the "kissing bug." Although the pathogen can also be transmitted to humans by blood transfusion,[3] congenitally,[4] by oral ingestion,[5] and by organ transplantations,[6,7] more than 80% of all human cases are caused by vector-borne transmission.[8]

In the United States, Chagas disease exists almost exclusively in animals.[9] Only 5 autochthonous insect-borne cases in humans have been reported in the southwestern United States.[10,11] The first autochthonous insect-borne case of Chagas disease in the United States was reported by Woody and Woody in 1955.[11] Trypanosomes were discovered in the blood of a 10-month-old girl from Corpus Christi, Texas.[11] One of the suggested reasons for the low incidence of human Chagas disease cases in the southwestern United States is differences in the resting and blood feeding (sylvatic and peridomiciliary) habitats of the insect vectors.[12]

Although the reported incidence of human Chagas disease in the United States is relatively low, North American strains of *T. cruzi* have been proven to be virulent among various mammals exposed to triatomine-triatomine species in nature.[12] Some of the common mammalian species naturally infected with *T. cruzi* are raccoon (*Procyon lotor* (Linnaeus)), wood rat (*Neotoma micropus* Baird), armadillo (*Dasypus novemcinctus*

(Linnaeus)), and domestic dog (*Canis familiaris* (Linnaeus)).[13,14] Many dog breeds have been infected and suffered mortality because of this infliction.[14] Twelve species of triatomines are known to occur in the southern half of the United States.[9] Seven of the triatomine species including: *Triatoma gerstaeckeri* (Stal), *T. indictiva* Neiva, *T. lecticularia* (Stal), *T. protracta* (Uhler), *T. rubida* (Uhler), and *T. neotomae* Neiva , *T. sanguisuga* (Leconte) have been routinely collected from Texas.[14] The overall infection rate in Texas has been found to be higher than in any other state where *T. cruzi* has been reported.[12] The *T. cruzi* infection rate among triatomine vectors in Texas has been reported as more than 50%.[15]

Chagas disease has proven to be a significant veterinary health threat to the military working dogs (MWDs). In late 2006, US Army veterinarians assigned to the Military Working Dog Center Veterinary Services, Lackland Air Force Base, San Antonio, TX, began to observe an increase of Chagas disease cases in the MWD population. Prior to this, only a few sporadic cases were identified through serology and clinical diagnosis. In 2007, veterinarians began to see an increase in the number of Chagas cases diagnosed. A cross-sectional serological study was conducted at one of the kennel facilities. It was demonstrated that a seroprevalence rate of 8% (24) of the dogs were positive for *T. cruzi* antibodies (S. Baty, written communication, June 30, 2007). This number was comparable to the number reported for stray dogs tested along the US border with Mexico.[9] In 2009, several MWDs were returned to the Military Working Dog Center with reported clinical symptoms of cardiac issues further diagnosed as Chagas disease. These dogs were supporting various units deployed to the Iraq theater of operations. As a result of evacuating the MWDs, these units were left without explosive detection dogs, thus leaving the units more vulnerable to attacks using improvised explosive devices. These findings have recently brought a new sense of urgency to better understand the Chagas disease vector. The purpose of this field study was to (1) survey *Triatoma* spp. found on Lackland Air Force Base, Camp Bullis, Medina Annex, and Fort Sam Houston; (2) develop *Triatoma* collection techniques; (3) characterize *Triatoma* habitats; and (4) determine relative incidence of *T. cruzi* in field collected specimens.

MATERIALS AND METHODS

Study Area

Five sites were selected based on proximity to potential host and habitat characteristics as indicated in the literature[9,12,13,16] (Figures 1 and 2).

Site 1. Medina Woods, N29° 23′ 07.97″ W98° 40′ 55.76″ (Figure 1). The Medina Training area consists of woodland habitat (oak, mesquite forest and open range land) located at the northeastern edge of the Medina Annex (Figure 2A).

Site 2, The Medina Kennel, N29° 23′ 13.71″ W98° 39′ 57.46″ (Figure 1). The Medina Kennel is a fixed site facility that houses approximately 800 dogs (Figure 3A).

Site 3, Transportation Security Administration (TSA) puppy breeding program kennels (N29° 24′ 06.96″ W98° 37′ 07.02″) are located on the northeastern side of Lackland Air Force Base (Figure 1). It is located south of Wilford Hall Medical Treatment Facility and is proximate to a wooded drainage area. Both kennel sites have several floodlights operating during the night.

Site 4, Western side of Camp Bullis, N29° 40′ 24.45″ W98° 36′ 23.74″ (Figure 1). Camp Bullis is located in the Texas Hill country. Predominate vegetation consists of oak, juniper, mesquite, prickly pear cactus, yucca plants, and open range (Figure 2B).

Site 5, Fort Sam Houston, N29° 27′ 38.28″ W98° 25′ 26.70″ (Figure 1). The site is located on the northeastern side of the installation between the golf course and the horse stables.

Field Collection Techniques

Triatomine bugs were collected from April to August 2011 at the study sites using 2 techniques.

- Excavation of dead wood. The wood excavation technique involved identifying likely triatomine harborage such as a sheltered position near a potential host nesting/resting location. A visual search was conducted at the harborage site. Sites examined included hollow or rotten logs, cactus plants, dead yucca plants, and other debris piles near burrows (Figure 2).
- Dog kennels surveillance. Dog kennels were inspected daily by kennel personnel and kissing bugs (Figure 3B) were collected using forceps and placed into 50 ml collection tubes with holes drilled in the tube cap for aeration.

Rearing, Preparation of Fecal Pools, and Species Identification

Specimens were maintained in the insectory by methods described by Durvasula and Taneja.[17] Insects were blood fed in vitro using a glass blood feeder[18] (Figure 4A) and defibrinated rabbit blood (less than 7 days old) (Lampire, Pipersville, PA). The blood was kept at 37°C using Super RMT LAUDA hot water circulator (LAUDA Brinkman, Delran, NJ). Each blood fed insect was

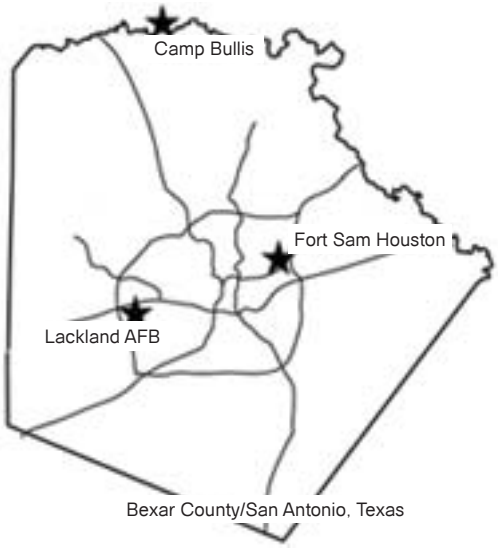

Figure 1. Map and aerial imagery of the 3 military installations in and around San Antonio, Texas, where specimens of triatomine bugs were collected, April through August 2011.

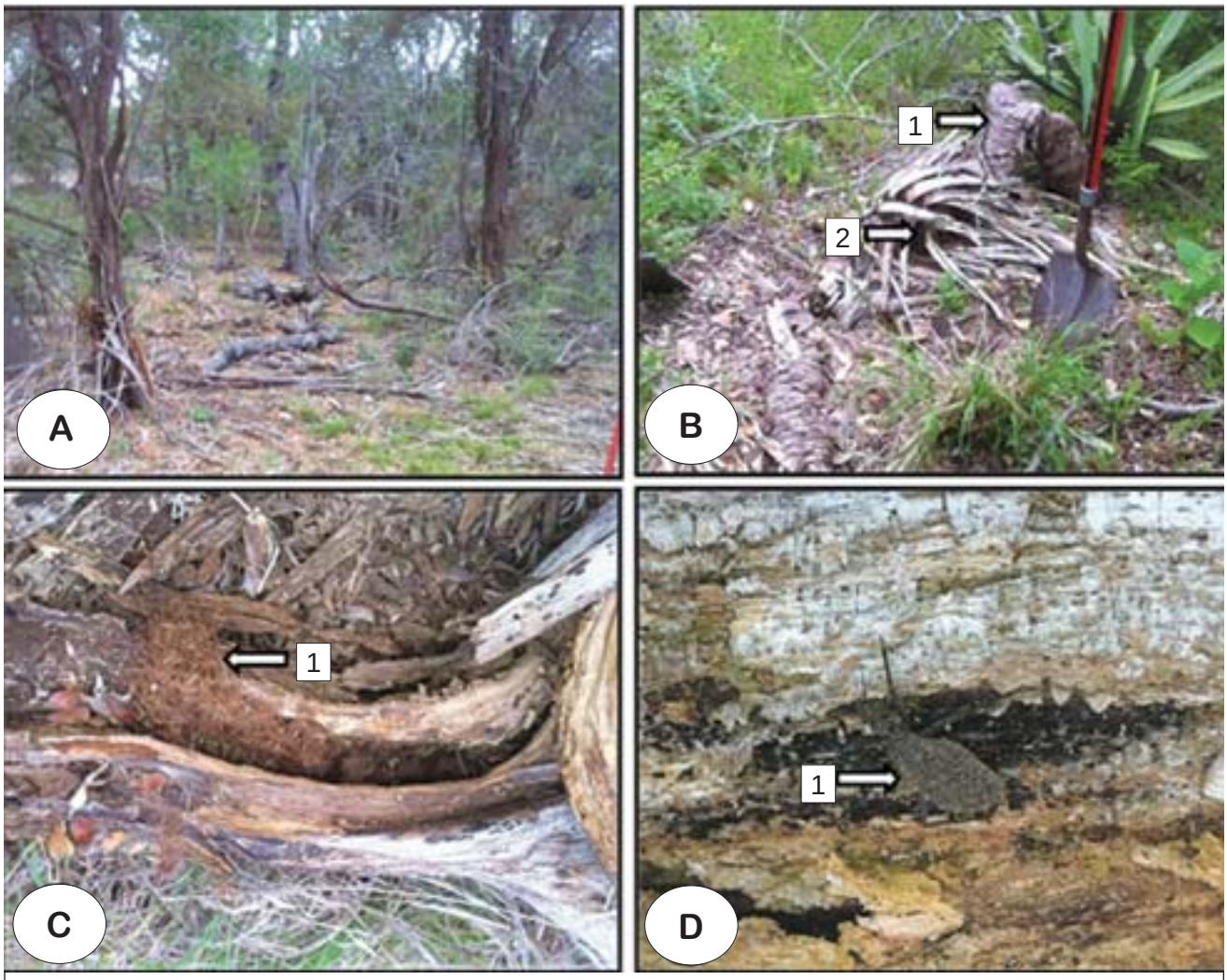

Figure 2. Collection habitat for triatomine bugs. Panel A, Medina Woods habitat; Panel B, dead Yucca plant (1) near a rodent burrow (2) from where triatomine bugs were collected at Camp Bullis; Panel C, hollow tree trunk with rodent nesting material (1); Panel D, *Triatoma* nymph (1) inside a hollow tree.

isolated individually in a 25 cm^2 cell culture flask, with canted neck and 0.2 µm vent cap (Corning Flask, Lowell, MA) (Figure 4B). The flasks were kept humid in plastic boxes with wet paper towels. Triatomines were identified using taxonomic keys by Lent and Wygodzinsky[19] (Figure 5). A database was maintained for all the specimens brought into the laboratory.

Detection of *T. cruzi* Infection by Traditional Polymerase Chain Reaction

Sample collection. Fecal matter from triatomine bugs was collected by plastic shaft with Dacron tip wetted with M4RT media liquid (Remel, Lexena, KS). Dacron tips were placed in 2.5 mL microtubes with 500 µL aliquoted liquid media and subsequently frozen at -20ºC until testing.

Sample processing. Aliquots used were subjected to one freeze/thaw cycle. From each sample, 140 µL was aliquoted for nucleic acid extraction. Specimen extractions were performed using the Qiagen QiaAmp Viral RNA Mini Kit (Qiagen Inc, Valencia, CA) according to manufacturer's minispin extraction recommendation. Feces from a subsample of the collected bugs (uninfected/infected) were checked for the presence and absence of live *T. cruzi* by magnification (×400) using a compound microscope ((Figure 4C, D) Table 1).

Primers. Primers used for amplification by traditional PCR of *T. cruzi* were:
primer set TCZ1/TCZ2:
forward primer TCZ1, 5′-CGAGCTCTTGCCCACAC GGGTGCT-3′

reverse primer TCZ2, 5′-CCTCCAAGCAGCGGATAG TTCAGG-3′)[20]

primer set S36/S36: forward primer S35, 5′-AAATAA TGTACGGGKGAGATGCATGA-3′
reverse primer S36, 5′-GGGTTCGATTGGGGTTGGT GT-3′).[2,21]

DNA amplification. The PCR was performed in 25 µl reaction mixtures consisting of 7.45 µl PCR grade water (Roche Diagnostics, Mannheim, Germany), 5 µl 5x Q-Solution (Qiagen, Valencia, CA), 5 µl GoTaq Flexi (Promega, Madison, WI), 1.5 mol $MgCl_2$, 0.5 µl deoxynucleoside triphosphates, and 0.15 µl of each primer (Integrated DNA Technologies, Coralville, IA). Reaction conditions (iCycler BioRad, Hercules, CA), for TCZ1/TCZ2 primers: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of amplification at 94°C for 20 seconds, 57°C for 10 seconds, and 72°C for 30 seconds, a final extension at 72°C for 7 minutes, then final hold at 4°C. Conditions for the S35 and S36 primers were processed under the following conditions: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of amplification at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for one minute, then a final extension at 72°C for 10 minutes, and held at 4°C until analysis.

The completed DNA reactions were analyzed by gel electrophoresis made with 2% agarose (Fischer Scientific, Fair Lawn, NJ) in 1X Tris-Borate-EDTA Buffer (Sigma-Aldrich, St Louis, MO) solution. Each sample set was run with a negative (without DNA) and positive control (with *T. cruzi* strain). Positive controls were acquired from the Department of Defense Food and Animal Diagnostics Laboratories at Fort Sam Houston, TX

Table 1. Distribution of *Trypanosoma cruzi* infection in different species of triatomine bugs collected at 3 US military installations in San Antonio, Texas, April to August 2011.

Location	Species	PCR Results	No. Tested	Percentage Infected	Visual Validation
Camp Bullis	*T. gerstaeckeri*	Not Infected	21	–	3
	T. indictiva	Not Infected	11	–	1
	T. lecticularia	Not Infected	1	–	
	T. sanguisuga	Not Infected	10	–	1
		Totals	43		5
Ft. Sam Houston	*T. gerstaeckeri*	Not Infected	2	–	1
		Totals	2		1
Lackland Air Force Base					
Medina Woods	*T. gerstaeckeri*	Not Infected	11	–	3
	T. gerstaeckeri	Infected	2	15.4	1
	T. indictiva	Not Infected	1	–	
	T. sanguisuga	Not Infected	22	–	2
	T. sanguisuga	Infected	3	12.0	1
		Totals	39		7
Medina Kennel	*T. gerstaeckeri*	Not Infected	9	–	4
	T. gerstaeckeri	Infected	10	52.6	10
	T. lecticularia	Not Infected	1	–	
	T. sanguisuga	Not Infected	4	–	
		Totals	24		14
TSA Puppy Kennel	*T. gerstaeckeri*	Not Infected	2	–	2
	T. gerstaeckeri	Infected	3	60.0	3
		Totals	5		5
Totals of tested specimens			113		31

TSA indicates US Transportation Security Administration.

RESULTS

A total of 140 triatomine-triatomine bugs representing 4 species (*T. gerstaeckeri*, *T. sanguisuga*, *T. lectularia*, and *T. indictiva)* were collected during this study (Table 2). The most prevalent triatomines collected were *T. gerstaekeri* (49%) and *T. sanguisuga* (39%). *Triatoma gerstaeckeri* was collected at all 5 study sites, while *T. sanguisuga* was only collected at 3 study sites. The majority of *T. gerstaeckeri* were collected at Camp Bullis (37%) and Medina Kennel (35%). All life stages were collected for *T. gerstaekeri* and *T. sanguisuga*. Four life stages (adult, 5th, 4th, and 3rd instars) were collected for *T. indictiva* and only adult collections resulted for *T. lectularia*. Only adults were collected at both kennel sites. Woodland collections from Camp Bullis and Medina resulted in collections of all life stages for *T. gerstaeckeri*.

Of 113 specimens tested using PCR for *T. cruzi*, 16% were positive. *Triatoma gerstaekeri* (25%) and *T. sanguisuga* (8%) were the only species that tested positive for *T. cruzi*. Infected bugs were only collected at 3 (Medina Woods, Medina Kennel, and TSA) of the 5 collection sites. The TSA kennel site had the highest occurrence (60%) of infection. However, only 5 bugs were collected. Medina kennel was the site with the second highest occurrence (53%) of infection. Only 2 life stages (adult and 5th instar) were positive for *T. cruzi*.

Figure 3. Dog kennel habitat at Lackland Air Force Base. Panel A, area between backside of dog kennels from where live triatomine bugs were frequently collected. Panel B, a triatomine bug resting on the concrete wall of a kennel.

Table 2. Distribution of species composition of triatomine bugs collected at 3 US military installations in San Antonio, Texas, April to August 2011.

Location	Species	Number	Percentage
Camp Bullis	*T. gerstaeckeri*	26	49.0
	T. indictiva	12	22.6
	T. lecticularia	1	1.9
	T. sanguisuga	14	26.4
	Total	53	
Ft. Sam Houston	*T. gerstaeckeri*	2	100
	Total	2	
Lackland Air Force Base			
Medina Woods	*T. gerstaeckeri*	13	27.1
	T. indictiva	1	2.1
	T. sanguisuga	34	70.8
	Total	48	
Medina Kennel	*T. gerstaeckeri*	25	78.1
	T. lecticularia	1	3.1
	T. sanguisuga	6	18.8
	Total	32	
TSA Puppy Kennel	*T. gerstaeckeri*	5	100
	Total	5	
Total Collected		140	

TSA indicates US Transportation Security Administration.

COMMENT

This study provided an updated status on the distribution and infection prevalence of triatomine species on 3 military installations in San Antonio, TX. Pippen[12] conducted the last comprehensive Chagas disease survey of this kind in the 1960s. From 1965 to 1966, Pippen conducted an extensive Chagas disease study on Lackland Air Force base. He collected over 386 specimens using 2 collection methods: wood rat dwelling inspection and black light trapping.[12] Pippin inspected 142 wood rat dwellings and collected 229 triatomines, of which the majority were *T. sanguisuga* and *T. gerstaekeri* nymphs. Of the specimens collected from the wood rat dwellings, 30% were infected with *T. cruzi* like organisms. While *T. sanguisuga* nymphs were the most prevalent specimens collected, *T. gerstaekeri* nymphs had a higher infection rate for *T. cruzi*.[12]

The triatomine distribution in our study was similar to Pippins' results. However, infection rates were different. This difference was most likely due to variance of collection techniques. While Pippin concentrated on wood rat dwellings, the majority of our specimens were collected inside the dog kennels (Figure 3). This collection technique appeared to be biased for adult specimens. Immature kissing bugs were never observed during collection at the kennel sites. Based on observed triatomine activity in the wood line near the kennels, we believe that adult kissing bugs flew to the kennels to feed. Furthermore, environmental conditions (short grass, roads between wood lines, and kennel daily cleaning regimen with high pressure water) at the kennel are not conducive for the establishment of triatomine colonies. Our results are similar to recent studies conducted in other areas of Texas.[9,12-15] In a study conducted by Kjos et al,[14] higher infection rates were observed in dog kennels in a domestic setting when compared to sylvan settings.

Our results indicate that the area with the highest *T. cruzi* infection rate is in and around the military working dog kennels. This is consistent with clinical observations at the MWD Center. Dogs observed at the MWD Center have exhibited multiple clinical signs. Canines affected with Chagas disease develop either acute or

chronic disease. Generally, dogs develop signs characterized by right-sided heart failure and cardiac arrhythmias.[22] Acute myocarditis, such as sudden collapse and death of a previously normal dog was observed in one case. Other symptoms associated with acute cardiac issues include lethargy, pale mucous membranes with slow capillary refill time, weak pulse, tachyarrhythmias, and respiratory arrest. Additionally, other clinical symptoms in dogs that do not die suddenly will exhibit a generalized lymphadenopathy, diarrhea, weight loss due to anorexia, fever, hepatosplenomegaly caused by the right-sided heart failure, ascites, and some neurological signs characterized by pelvic limb ataxia.[23] Dogs which overcome the acute symptoms and become chronic survivors will develop further cardiac issues to include arrhythmias which can be exacerbated by exercise or hard work on the battlefield. As the chronic myocarditis progresses, the heart muscle undergoes a progressive cardiac degeneration and dilation. The result is a bilateral enlarged heart with flaccid thin walls that exhibit abnormal electrical impulses and arrhythmias on an electrocardiograph, along with possible respiratory distress. These cases can often be confused with chronic dilative cardiomyopathy observed in other large breeds of dogs.

Effective surveillance for Chagas disease vectors is essential for the development of a control program. Few studies have been published regarding the ecology of North American Triatomines. Most research efforts have been conducted in endemic countries (Mexico, Guatemala, Brazil, and Peru) in Central and South America. Surveillance techniques in these areas yielded large numbers of triatomines. This is mainly due to the difference in abundance, behavior, and biology. The main South American species responsible for Chagas

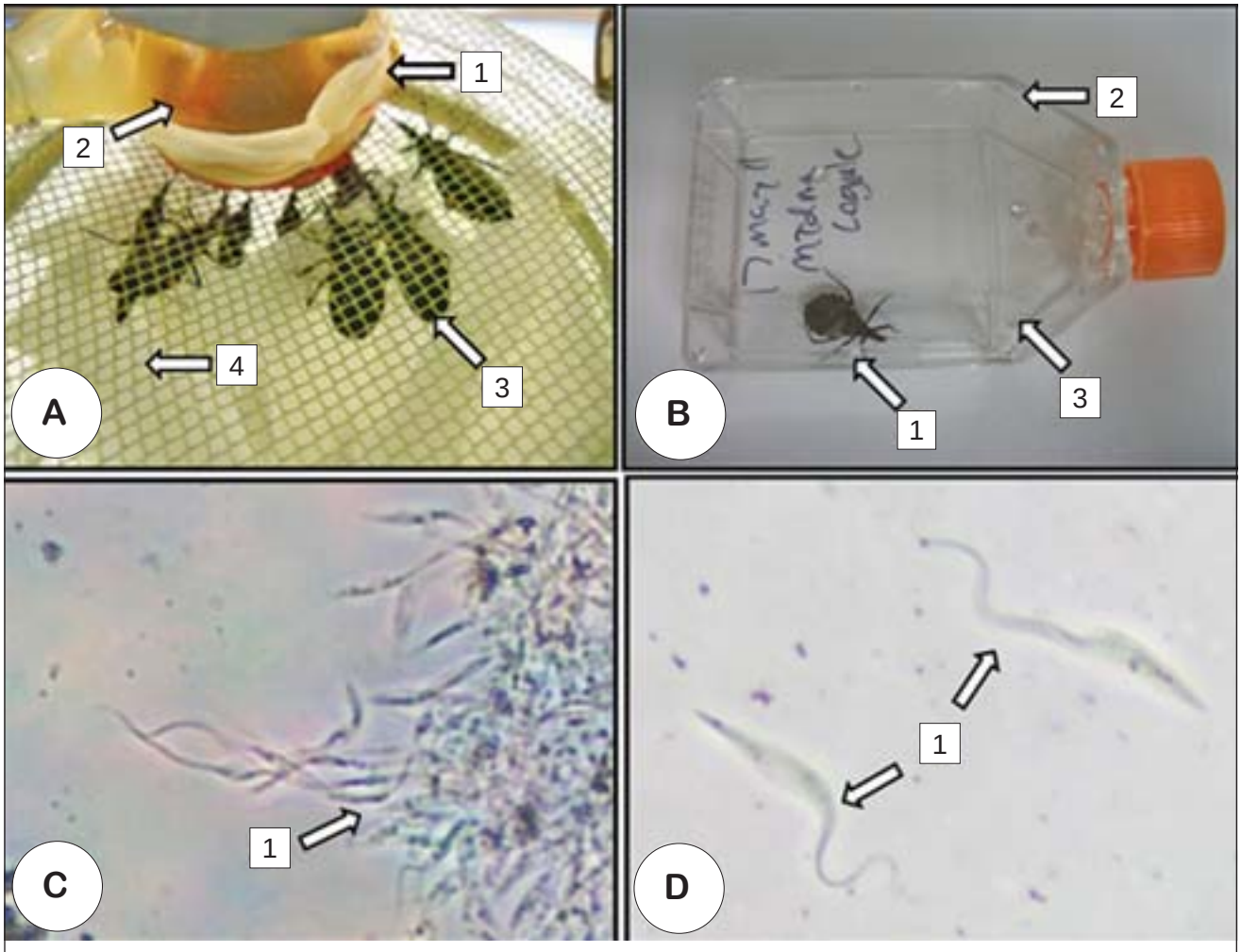

Figure 4. Various experimental components of *T. cruzi* infection procedure. Panel A, an in vitro blood feeding method using a hog intestine membrane (1) stretched on a glass feeder (2) and *T. gerstaeckri* (3) feeding through a netting (4) on defibrinated rabbit blood. Panel B, isolation of a blood fed nymph (1) in a cell culture flask (2) to obtain infected urine (3) and feces. Panel C, (1) contents of an infected midgut showing epimastigote stage. Panel D, (1) actively motile metacyclic trypomastigote stage from the feces of an infected triatomine bug.

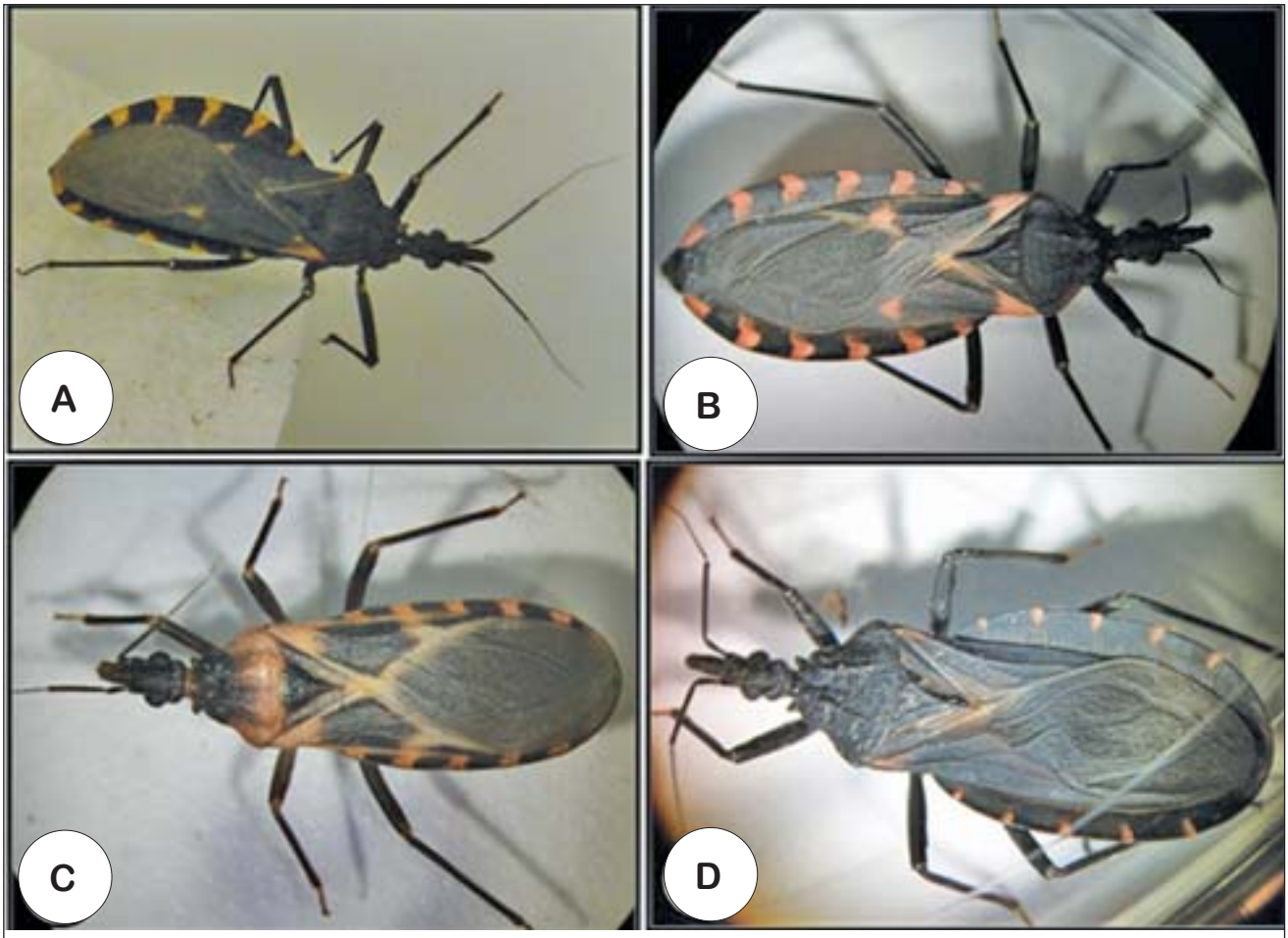

Figure 5. Four species of triatomine bugs that were collected during field surveillance. Panel A, *T. gerstaeckeri* female. Panel B, *T. sanguisuga female*. Panel C, *T. lecticularia* male. Panel D, *T. indictiva* male.

transmission (*T. infestans* (Klug)) was more urban and cohabitated with humans. North American species are more elusive and mainly sylvatic. The 2 most common techniques cited in the literature are light trapping and wood rat nest excavation. These two collection methods rarely give a reliable indication of population size.

The wood excavation method was the most effective surveillance technique in this study because we collected all life stages for 4 different *Triatoma* species. During the peak of the summer, this method proved to be consistently effective for collecting large numbers of specimens. However, this method is labor intensive and requires skill for habitat acquisition.

Possible oral transmission of Chagas disease poses a challenge for the control of Chagas vectors at the MWD Center. Conventional pesticide applications would not be effective due to the cleaning regimen and could possibly expose the dogs to dead or dying insects. Control of the reservoir hosts (rodents) in the woodland habitat with rodenticides could lead to secondary poisoning of the military working dogs.[24,25]

A solution to the problem would be the use of a systemic insecticide for the control of the triatomine vectors. Systemic insecticides attack insects directly through their living food sources without harming the host species, and can be impregnated into a grain bait to treat the rodents and any other potential reservoir hosts in the wooded areas around the MWD Center. Pesticides with low mammalian toxicity are available, and should pose no unreasonable threat to the military working dogs.[26] Kaput Rodent Flea Control Bait (EPA Reg. No. 72500-17) is an imidicloprid systemic bait well suited for this application. This product can be used in accordance with the Federal Insecticide, Fungicide, and Rodenticide Act,[27] Section 2ee, by treating the site specifically as described on the product label.

Field collected specimens were mainly collected in habitats that included hollow logs of different species (live

oak and cedar) and dead dry yucca plants. Triatomines were rarely found in rodent nests. The most common habitat conditions included a hollow log over or near an active rodent burrow. It appeared that the kissing bugs entered the nest to feed, and then retreated to harborage for protection. Various arthropods are often found cohabitating inside the logs with the kissing bugs (scorpions, wood boring beetles, pill bugs, and centipedes). Acorns, rodent droppings, and nesting material were also indicators for positive habitat association. If fire ants were present near or in the log or burrow, no kissing bugs were found.

In summary, Chagas disease continues to pose significant health risks to the military working dog population in San Antonio. In order to develop an effective control method, further research is warranted to understand both biology and ecology of local triatomine species.

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A Field-expedient Method for Detection of Leptospirosis Causative Agents in Rodents

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ABSTRACT

We have developed a thermal-stable, pathogenic *Leptospira* TaqMan PCR assay intended to support pathogen surveillance in reservoir populations. The assay is packaged specifically for use with a portable, ruggedized, real-time PCR thermocycler. Limit of detection was established at ≤100 fg (20 organisms). Sensitivity and specificity were 100% concordant with conventional PCR results using a broad test panel of human pathogenic and nonpathogenic *Leptospira*, genetic near neighbors, and clinically significant organisms. In blind testing using a panel (n=50) of pathogenic *Leptospira* infected and noninfected *Rattus* species samples, assay sensitivity results were 100% concordant with conventional PCR. Tests performed under field conditions using wild-collected rodent kidney extracts demonstrated the mobility of the system. During field evaluation, samples were processed and analyzed in 3 hours. Thermal stabilized reagents allowed transportation, storage, and analyses under ambient temperatures. The system provides a promising aid in leptospirosis control programs.

Routine biosurveillance and outbreak response systems are important public health tools which can facilitate prevention of infectious diseases through early detection and identification of pathogen emergence and mitigation of outbreaks through focused and timely response efforts. Rodent-borne zoonoses are a significant cause of morbidity and mortality worldwide and rapid recognition is critical to minimizing disease transmission at the local level and the spread of pathogens globally.

Leptospirosis is one of the most widespread zoonotic diseases in the world.[1-3] Genus *Leptospira* bacteria are classified into 17 species and over 200 serovars comprised of pathogenic, opportunistic, and nonpathogenic organisms.[4] *Leptospira* are transmitted by infected wild and domestic animals with rodents recognized as the most significant reservoir. Transmission to humans is by contact with infected urine in water, soil, and surfaces and through direct contact with infected animals.

The absence of a licensed vaccine against *Leptospira* and limitations in leptospirosis diagnostics and treatment drive the need for efficacious prevention and control. Surveillance of potential sources of *Leptospira* transmission serves a valuable role in leptospirosis risk assessment. Leptospirosis prevention is dependent on control of infected animals and awareness and elimination of contaminated environmental sources. To most efficiently make use of finite surveillance resources risk assessment activities must be focused on likely transmission foci and the associated environment. Analyses and risk assessment conducted in a timely manner is critical to effectively implementing prevention and control resources in an outbreak or potential outbreak situation.

Disease outbreaks often occur in developing regions and coincide with natural disasters or in war-torn areas. It is under these conditions that rapid disease surveillance, efficacious risk assessment, and appropriate and efficient use of control resources are most critical. However, *Leptospira* reference methodology by microscopic agglutination test requires up to 3 weeks for culture incubation.[5-8] As such, real-time PCR can serve as a valuable aid in surveillance and provides promise in diagnostics. Rapid and highly sensitive and specific molecular-based detection tests have been developed, however, these technologies are designed for use in a fixed laboratory infrastructure and as such are not suitable for use under austere and extreme field conditions.[7-16] In situations of an underdeveloped, damaged, or totally absent infrastructure, disease surveillance must be conducted without access to laboratory facilities, electricity, or cold-chain resources. Disruption of transportation systems and power grid are some of the obstacles that drive the need for mobile and independently operating disease surveillance systems.

We have developed a highly sensitive and specific, thermal-stable, pathogenic *Leptospira* species (LPS) PCR detection assay formatted for use with mobile, autonomously operating, field-proven, real-time PCR instrumentation.[17-20] We describe a field-expedient method for

sensitive and specific detection of leptospirosis causative agents in rodents.

MATERIALS AND METHODS

Study Site. Field-evaluation was conducted at Buri Ram Province (14° 33′ 30″ N, 102° 55′ 30″ E), Thailand, 16 to 20 August 2010. The LPS PCR assay, nucleic acid preparation reagents, and PCR instrument were transported, stored, and sample preparation and analyses conducted under ambient temperature (25°C to 33°C) and humidity (80%-100%) conditions. Staff and equipment and supplies were transported by a van to the field site. The field laboratory was set up and system operations confirmed within 2 hours. The laboratory was established in a single room of a building without environmental control using 2 tabletops (each approximately 1 m^2). Sample preparation and analyses were conducted without provisions for spatial separation or containment.

Wild-caught Rodents. Field-evaluation of the LPS assay in vivo sensitivity was conducted using a test panel of wild-caught rodent kidney tissue extracts (n=36). Sampling was conducted for 2 nights in the rice field and forest around the rural villages in 3 study sites (Chamni (14° 47′ 18″ N, 102° 50′ 30″ E), Khu Mueng (15° 16′ 18″ N, 103° 0′ 6″ E), Lahan Sai (14° 24′ 42″ N, 102° 51′ 36″ E) districts of Buri Ram province). In each site, rodent habitats were identified, and small wire live-traps (14 cm wide, 14 cm high, 30 cm long) specially fitted for rodents were set. A mixture of banana and snail was used for bait. The traps were placed in the evening (between 4 PM and 5 PM) and collected early the following morning (7 AM to 8 AM). Captured rodents were euthanized by carbon dioxide overdose.[21] Rodent kidneys and spleens were aseptically removed and extract prepared as described below. *Rattus rattus* was the most prevalent species (subspecies identification was not made). Sample extracts were transported on dry ice from the field site to the Armed Forces Research Institute of Medical Sciences laboratory for confirmation testing using a well established *Leptospira* gyrase subunit B conventional PCR assay.[15] All animal activities were approved by the Institutional Animal Care and Use Committee and conducted in an Association for Assessment and Accreditation of Laboratory Animal Care International (Frederick, MD) accredited facility and in compliance with the Animal Welfare Act (7 USC §§ 2131-2156) and other federal statutes and regulations involving animals.

Preparation of Nucleic Acid Extract. Total nucleic acid extracts were prepared from bacterial cultures, viral cultures, and rodent kidney and spleen tissues using QIAamp DNA Mini kit, QIAamp viral RNA Mini kit (QIAGEN Inc., Valencia, CA), and DNA preparation kit (Wizard Genomic DNA Purification Kit (Promega Corp, Wisconsin)) respectively. *Leptospira* DNA was quantified using the Qubit fluorometer (Life Technologies, Grand Island, NY) following the manufacturers' instructions. Extracts were stored at -70°C.

Design of PCR Probe and Primer Oligonucleotides. The LPS TaqMan PCR assay primer and probe oligonucleotide sequences may be requested from the primary author. Oligonucleotides were designed de novo by eye targeting a 132 base pair (bp) sequence of the gene encoding *Leptospira interrogans* serogroup Australis major outer membrane protein (lipl32); GenBank accession number: AY609325.1. Oligonucleotide sequences of human pathogenic *Leptospira* species were selected considering the following guidelines:

- amplicon length=75-150 bp
- oligonucleotide length=18-30 bases
- guanine and cytosine content=30%-80%
- primer melting temperature (Tm)=63°C to 67°C
- probe Tm 8°C to 10°C higher than primer Tm
- probe placement relative to primers (proximal)
- avoidance of runs of identical nucleotides to prevent mismatching and nucleotide complementarities to prevent secondary structure (hairpin-loop) formation and oligonucleotide dimerization.

Melting temperatures were quantified and the absence of significant secondary structure formation and dimerization were confirmed with PrimerExpress software (PE Applied Biosystems, Foster City, California). Primer and probe sequence heterology with genomic sequences of closely related species through diverse genera were validated by BLAST (Basic Local Alignment Search Tool) database search.[23] Primer and probe synthesis and quality control were conducted by a commercial vendor (Idaho Technology, Inc, Salt Lake City, Utah). The TaqMan probe contained 2 fluorigenic labels, a 5′ reporter dye (6-carboxyfluorescein (FAM)) and a 3′ quencher dye (6-carboxytetramethylrhodamine (TAMRA)) (Roche Molecular Diagnostics, Pleasanton, California).[24,25]

Polymerase Chain Reaction. Wet reagent LPS PCR assay optimization was conducted on the "Ruggedized" Advanced Pathogen Identification Device (R.A.P.I.D.) (Idaho Technology, Inc (ITI), Salt Lake City, Utah). Primers and probe were optimized with R.A.P.I.D. wet reagents and the optimum condition was 5 mmol/L $MgCl_2$, 400 nmol/L primers, 100 nmol/L probe. The master mix contained LPS 400 nmol/L forward and reverse primers, 100 nmol/L TaqMan probe, 200 µmol/L each dNTP, 5 mmol/L $MgCl_2$, 1×PCR buffer, 1×stabilization buffer, and Taq Polymerase:Ab: Enzyme diluent (1:1:10.5).

The optimal LPS PCR master mix formula was used for LPS assay preparation and production conducted by an ITI proprietary process. Freeze-dried LPS PCR master mix reagents only required hydration and addition of sample template prior to analysis. Assays were prepared according to the manufacturer's (ITI) instructions. A positive template control reaction was prepared using *L interrogans* serovar Bangkok at a total concentration of 1 pg template. Negative template control reactions were prepared using PCR grade water. A R.A.P.I.D. standardized PCR thermal cycling protocol consists of an initial DNA denaturation at 95°C for 3 minutes, and PCR for 45 cycles at 95°C for 0 seconds for template denaturation (sinusoidal temperature cycling) and 60°C for 20 seconds of combined annealing and primer extension.

Linearity and Limit of Detection. The linearity of the LPS freeze-dried assay was assessed in order to determine the amplification efficacy and efficiency of the PCR. These data were used to estimate limit of detection (LOD). The estimated value served as the starting point for further evaluation of LOD by replicate sample test. The correlation coefficient (R^2) of standard DNA concentrations was used to establish linearity. The slope was used to calculate amplification efficacy and efficiency using the formulas:

$$\text{Efficacy} = -1 + 10^{(-1/\text{slope})} \qquad \text{Efficiency} = 10^{(-1/\text{slope})}$$

The LOD was estimated using a standard curve produced by plotting critical threshold (Ct) values versus the logarithm of serial dilutions of *L borgpetersenii* serogroup Ballum serovar Ballum at 10 ng to 1.0 fg genomic DNA per reaction volume. The Ct values of each log DNA concentration were measured in 2 replicates. Least-squares regression analysis (performed by the R.A.P.I.D. software) plotted Ct as a function of DNA concentration. The R.A.P.I.D. software automatically calculated "best-fit" of the regression and a standard curve was established, the linear relationship between ΔPCR cycle number and ΔDNA concentration. The R^2 value was automatically adjusted near or at unity by the R.A.P.I.D. software.

The LOD was estimated as the template concentration at the lowest Ct value above background. The estimated LOD was used to conduct replicate sample testing (n=20). Replicate sample testing was conducted by 3 operators.

Rodent extracts and *Leptospira* Reference Strains. A test panel of well characterized rodent kidney extracts from sample archives was prepared consisting of 30 pathogenic *Leptospira* infected tissue extracts and 20 noninfected extracts. Kidney tissue extracts were previously prepared and confirmed positive for pathogenic *Leptospira* species by *Leptospira* gyrase subunit B conventional PCR.[8] Extracts were archived at -70°C. Prior to LPS assay sensitivity testing, template quality was confirmed using *Leptospira* gyrase subunit B conventional PCR.

Validation testing of LPS PCR assay sensitivity and specificity were conducted using a diverse panel of 24 reference serovars of *Leptospira* species consisting of 22 pathogenic and 2 nonpathogenic serovars (Table 1). Reference strains were obtained from the Department of Leptospirosis Laboratory, National Institute of Animal Health, Thailand. Cultures were grown in Ellinghausen-McCullough-Johnson-Harris medium (Difco Laboratories, Detroit, Michigan) and maintained by weekly subculture at 30°C following established methodology.[22] Reference sample quality was confirmed using *Leptospira* gyrase subunit B conventional PCR.

Non-*Leptospira* Organisms: Specificity Test Panel. Specificity testing included a panel of a well characterized nucleic acid extracts consisting of non-*Leptospira* genetic near neighbors, clinically significant organisms, and *R. rattus* and human DNA (Table 2). Organisms harboring RNA genomes underwent reverse transcription to produce genomic cDNA for testing. The intent of cDNA testing was to confirm exclusion of potential laboratory introduced crossover contaminates.

Data Analysis. Sample identification and specifications were entered electronically in the R.A.P.I.D. operating system run protocol. Analyses and results were automatically archived. The criterion for a positive result was a significant increase in fluorescence over background levels, ie, Ct, defined by an algorithm provided in the R.A.P.I.D. analytical software. The Ct is defined as the first PCR cycle with significant fluorescence when normalized against background fluorescence. Samples with a Ct of $\geq$40 were considered negative, while samples with a mean Ct of $<$40 were considered positive by R.A.P.I.D. analyses.

RESULTS

Linearity. Linear regression analyses of the LPS freeze-dried assay using *L borgpetersenii* serovar Ballum concentrations ranging from 10 ng to 1 fg of total nucleic acid (2 replicates for each of eight 10-fold dilutions) demonstrated the robustness of the assay. Amplification was linear from 10 ng to 100 fg of template concentration. Slope and best fit of correlation coefficient (R^2) and error values were performed automatically by regression analyses software included in the software package of the R.A.P.I.D. operating system. Linearity was

Table 1. Reference strains tested by *Leptospira* pathogenic spp (LPS) PCR

Serogroup	Serovar	Strain	LPS Results* (mean Ct)†
Pathogenic *L. interrogans*			
Australis	Bratislava	Jez Bratislava	35.44
Autumnalis	Autumnalis	Akiyami A	33.82
Australis	Bangkok	Bangkok-D92	34.50
Bataviae	Bataviae	Swart	35.36
Canicola	Canicola	Hond Utrecht IV	35.47
Djasiman	Djasiman	Djasiman	35.31
Hebdomadis	Hebdomadis	Hebdomadis	36.22
Icterohaemorrhagiae	Icterohaemorrhagiae	RGA	35.18
Pomona	Pomona	Pomona	35.12
Pyrogenes	Pyrogenes	Salinem	35.16
L. borgpetersenii			
Ballum	Ballum	RATTUS SP 127	34.32
Javanica	Javanica	Veldrat Bataviae 46	34.89
Mini	Mini	Sari	34.09
Sejroe	Sejroe	M84	35.33
Tarassovi	Tarassovi	Perepelitsin	35.50
L. kirschneri			
Cynopteri	Cynopteri	3522 C	35.06
Grippotyphosa	Grippotyphosa	Moskva V	34.25
L. noguchii			
Louisiana	Louisiana	LSU 1945	34.82
Panama	Panama	CZ 214	34.99
L. weilii			
Celledoni	Celledoni	Celledoni	34.77
L. santarosai			
Shermani	Shermani	1342 K	35.67
L. inadai			
Manhao	Manhao	Li 130	38.23
Nonpathogenic *L. biflexa*			
Semaranga	Patoc	Patoc I	Negative
Andamana	Andamana	CH 11	Negative
L. meyeri			
Ranarum	Ranarum	ICF	Negative

*Pathogenic *Leptospira* sample population (n=22) mean Ct=35.16, SD=0.89, CV%=0.78.
†*Leptospira* strain mean Ct value represents duplicate testing at the LOD concentration (100 fg).

Table 2. Results of negative control testing.

Species	LPS Results
Human blood	Negative
Rodent blood (*Rattus rattus*)	Negative
Escherichia coli	Negative
Shigella flexneri	Negative
Shigella sonnei	Negative
Pseudomonas aeruginosa	Negative
Klebsiella pneumoniae	Negative
Enterobacter aerogenes	Negative
Staphylococcus aureus	Negative
Staphylococcus typhimurium	Negative
Streptococcus pyogenes	Negative
Bartonella doshiae	Negative
Plasmodium falciparum	Negative
Plasmodium vivax	Negative
Japanese Encephalitis Virus (cDNA)	Negative
West Nile Virus (cDNA)	Negative
Tembusu Virus (cDNA)	Negative
Dengue Virus Serotype 1 (cDNA)	Negative
Dengue Virus Serotype 2 (cDNA)	Negative
Dengue Virus Serotype 3 (cDNA)	Negative
Dengue Virus Serotype 4 (cDNA)	Negative

quantified at slope=3.378, R^2=1.00, and error=0.0613. *Leptospira interrogans* serovar Bangkok positive template control (PTC) reaction prepared at 1 pg concentration reported fluorescence at an average Ct value of 31.85 corresponding with *L borgpetersenii* serovar Ballum 1 pg concentration average Ct value of 32.03.

Limit of Detection. The LOD was estimated at ≤100 fg or ≤20 genome equivalent (ge) based on linear regression analyses results. A total of 60 replicate R.A.P.I.D. runs at 100 fg concentration *L borgpetersenii* serovar Ballum total nucleic acid template established the LOD at ≤100 fg (20 ge). Three operators running 20 replicates reactions each over a 2-day period achieved a replicate test score of 100% (60/60). Operator 1 mean (μ) Ct values were 35.02, SD=0.51, and percent coefficient of variation values (CV%)=1.45 where n=20, SE=0.11 and 95% confidence interval (CI)=34.80-35.24. Operator 2 mean Ct values were μ=35.38, SE=0.75, and CV%=2.11 where n=20, SE=0.17, and 95% CI=35.05-35.71. Operator 3 mean Ct values were μ=35.61, SE=0.63, and CV%=1.76 where n=20, SE=0.14, and 95% CI=35.33-35.89.

Sensitivity and Specificity Testing. In LPS assay sensitivity and specificity testing with *Leptospira* reference strains, sensitivity and specificity results were 100%

concordant with *Leptospira* gyrase B conventional PCR analyses. (Table 1). Twenty-five *Leptospira* reference strains consisting of 22 pathogenic serovars were positive by LPS assay analyses and 3 nonpathogenic serovars did not report fluorescence above background. All samples were tested in duplicate at a DNA concentration of 100 fg (1×LOD). Pathogenic *Leptospira* sample population Ct values were μ=35.16, SD=0.89, and CV%=0.78 where n=22, SE=0.19, and 95% CI=34.79-35.53. Nonpathogenic serovars from the panel tested at 1 pg and 100 pg DNA concentrations (100× and 1000×LOD) reported no fluorescence above background. Inhibition of PCR was not observed at 100 pg DNA concentration (1000×LOD) using 3 pathogenic *Leptospira* serovars: *L interrogans* serogroup Australis serovar Bangkok (Ct=15.98), *L interrogans* serogroup Australis serovar Bratislava (14.34), and *L weilii* serogroup icterohaemorrhagiae serovar Sarmin (Ct=33.29). A single anomalous result occurred, *L weilii* serovar Sarmin was detected at 1 pg (Ct=39.62) but did not report fluorescence at the 100 fg LOD level. This result was not included in the statistical analyses because *L weilii* serovar Sarmin sequence is 100% homologous with primer and probe sequences and as such probable experiment error is under assessment.

Archived and wild-captured rodent kidney tissue extracts tested by the LPS assay demonstrated 100% sensitivity compared to the *Leptospira* gyrase subunit B conventional PCR assay (Table 3). Using a test panel of 50 archived rodent tissue extracts, 30 *Leptospira* infected extracts were positive by LPS assay analyses and 20 noninfected extracts did not report fluorescence above background (Table 3). Sample preparation and blind testing were conducted under controlled laboratory conditions. *Leptospira* infected rodent extract Ct values were μ=29.50, SD=3.31, and CV%=10.98 where n=30, SE=0.60, and 95% CI=28.32-30.68.

In field evaluation using a test panel of 36 wild-captured rodent tissue extracts, 4 *Leptospira* infected extracts were positive by LPS assay analyses and 32 noninfected extracts did not report fluorescence above background (Table 3). Sample preparation and testing were conducted under field conditions. Wild-captured rodent extract Ct values were μ=34.34, SD=4.83, and CV%=23.36 where n=4, SE=2.42, and 95% CI=29.61-39.07.

Specificity Testing Using Negative Control Organisms. Specificity of the LPS assay was 100% concordant with a diverse panel of well characterized non-*Leptospira* organisms (Table 2). No cross-reaction occurred with human or *Rattus* species undiluted extracts from blood or kidney tissue, respectively. Ten common infectious disease agents and Total nucleic acid extract from 10 infectious disease agents and cDNA prepared from 7 viruses were tested at a concentration of 1000×LOD. No fluorescence above background was observed for all non-*Leptospira* organisms tested.

Throughout laboratory validation testing and field evaluation, PTC reactions reported fluorescence at the expected Ct value (≈32) and negative template control reactions did not report fluorescence above background.

COMMENT

Our results clearly show that the LPS assay is a robust, portable, highly sensitive, and specific test for the detection of pathogenic *Leptospira* species In evaluation with *Leptospira* infected rodent kidney extracts, the assay proved to be sensitive with no false negative or false positive results. The stability of the assay was evidenced by the reproducibility of PTC results. Use of the assay with the R.A.P.I.D. provided a highly mobile, standalone, real-time PCR analytic system for field-deployed rodent surveillance. During field evaluation, the system was configured and normal operations confirmed in less than 2 hours. Sample processing and analyses were completed in less than 3 hours. The system is unique in its ability to fill an important public heath role as it provides rapid pathogenic *Leptospira* detection capability under austere and extreme operating conditions.

Targeting transmission risk areas and identifying preventable conditions help focus control resources. Correctly collected and interpreted data on rodent infection rate and prevalence of contaminated environment integrated with other key transmission indicators such as confirmed leptospirosis cases (where epidemiological data is available), virulence of the circulating *Leptospira* serovar, rodent infestations and population densities, reproduction rate, terrain and climatic conditions, provide for efficacious transmission risk assessment. These data collected in a spatially focused and expedient manner, augment the predictive power of field surveillance allowing decision makers to dedicate control resources for focused application of animal abatement measures, treatment of habitat, and increased public awareness. The value of animal and environmental surveillance is enhanced by field-expedient detection capability.

Limitations in leptospirosis diagnostics must be addressed. Achieving a definitive diagnosis across both the acute and immune phases of leptospirosis is challenging because clinical symptoms are easily confused with those of other common diseases.[5,26] The treatment of leptospirosis can be enhanced by rapid and highly sensitive diagnostics.[5] Antibiotics are most effective when started by day 5 of disease onset and as such early diagnosis

Table 3. Results of rodent kidney tissue testing

Samples	No. Samples	No. True Pos	No. True Neg	LPS PCR Sensitivity	gyrB PCR Sensitivity
Archived rodent extract*	50	30	20	100% (30/30+0)(100%)	100% (30/30+0)(100%)
Wild-captured rodents†	36	4	32	100% (4/4+0)(100%)	100% (4/4+0)(100%)

*Archived rodent extract (n=30) mean Ct=29.5, SD=3.31, CV%=10.98.
†Wild-captured rodent extract (n=4) mean Ct=34.34, SD=4.83, CV%=23.36.

would be beneficial in the treatment of leptospirosis.[4] However, while rats may shed up to 10^8 spirochetes per ml of urine, leptospirosis patient sample concentrations present challenges in detection limit. The presence of *Leptospira* organism/DNA can vary from very low to high levels during the acute (2-7 days) and immune (0-30 days) phases of the disease depending on the seriousness of the infection.[27] Patient urine sample concentration of *Leptospira* ranges from 10^2 to 10^4 spirochetes per ml and the asymptomatic urinary range is 10^1 to 10^3 spirochetes per ml.[28] Blood sample concentration of *Leptospira* ranges from 10^1 to 10^5 spirochetes per ml.[29] Diagnosis of leptospirosis is usually retrospective because of the length of the time required for diagnosis by microscopic agglutination test (MAT) reference methodology.[5-8] The MAT and other agglutination-based tests have been developed for more rapid and convenient diagnostics, however, these methods have limitations in specificity.[30] An approved molecular-based human diagnostic test does not currently exist that does not require confirmation testing by *Leptospira* isolation and culture. Molecular-based methodologies describing direct detection from clinical samples are not currently well represented in the literature. It is our intent to transition the LPS assay to human diagnostic use. We will address challenges in achieving efficacious PCR-based leptospirosis diagnostics by enhancing the high sensitivity and specificity of the LPS assay procedurally, adapting specialized protocols to concentrate patient samples, and the development of extraction and PCR internal positive controls.

Our results show that the LPS TaqMan assay is a field-expedient method for sensitive and specific detection of leptospirosis causative agents in rodents.

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DISCLAIMER

Reference to trade name, vendor, proprietary product or specific equipment is not an endorsement, a guarantee or a warranty by the Department of the Defense or US Armed Forces, and does not imply an approval to the exclusion of other products or vendors that also may be suitable.

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Military Entomology in Operation Enduring Freedom, 2010-2011

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Since 2001, the United States has been engaged in Operation Enduring Freedom (OEF) in Afghanistan. Although vector-borne disease in Afghanistan does not present as high a risk in comparison to other areas into which the US military deploys, such as sub-Saharan Africa, it does present sufficient risk to adversely impact military operations. This article discusses the growth and state of US military entomological support to the Afghan theater of operations (ATO).

ENTOMOLOGICAL SUPPORT TO THE AFGHAN THEATER OF OPERATIONS

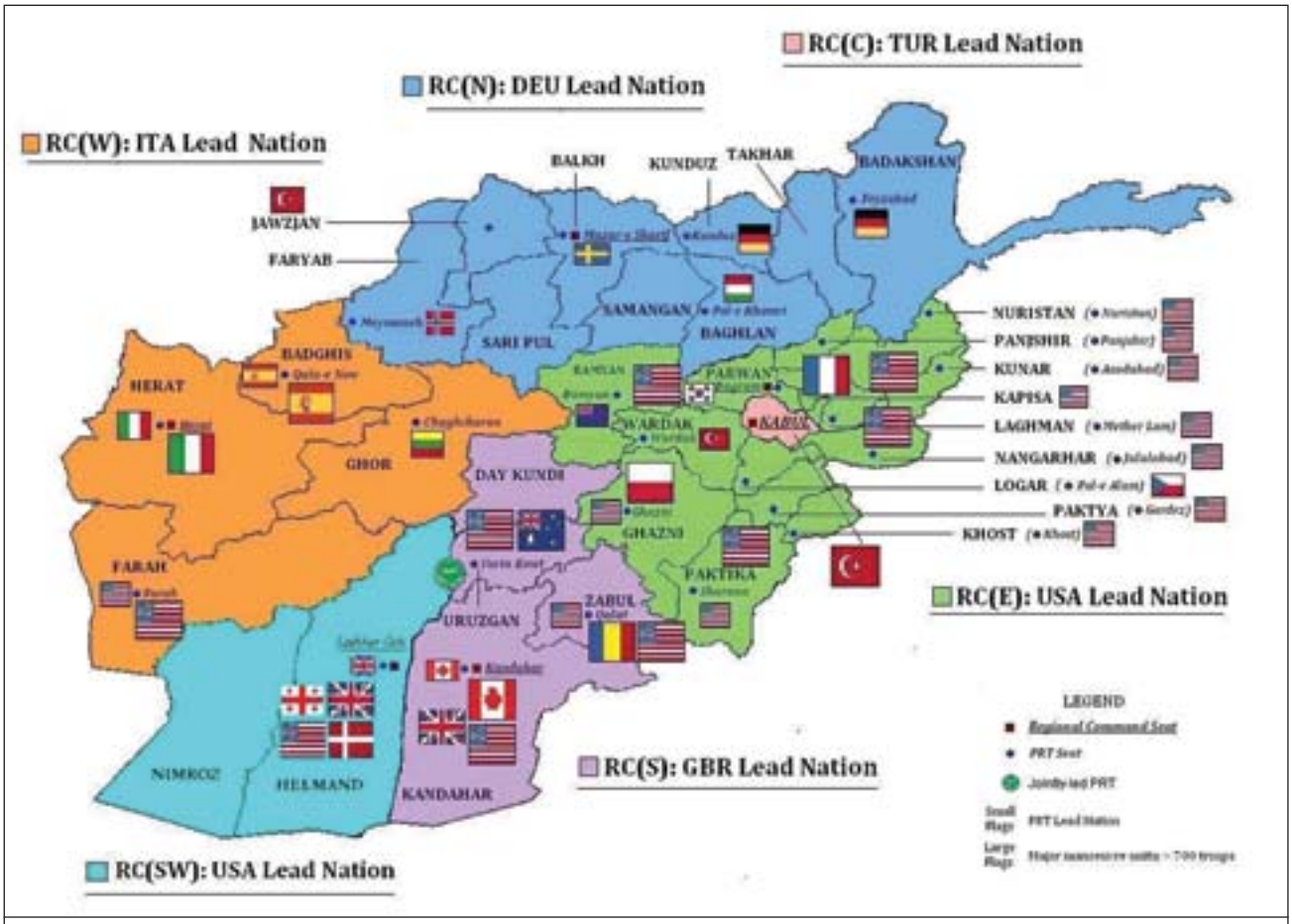
Providing entomological support to the Afghanistan theater of operations presents unique challenges to preventive medicine personnel, similar to those experienced in Iraq. Military and civilian infrastructure throughout the country remains poor, despite significant buildup and investment since 2001. Ground logistic routes are limited and security concerns often restrict movement not essential to direct support of combat operations and sustainment. Access to many US military camps in Afghanistan is primarily by air, and a number of locations are accessible only by air. Transporting equipment and pesticides further complicates the challenge of this operational reality. Consistent and comprehensive vector surveillance and disease reporting were improved over the years, however, coordination and oversight are required to identify pest and vector issues.

As a result of its diverse topography and climate, Afghanistan experiences significant variation in pest and vector issues. Each vector-related problem presents unique challenges that require careful coordination between base operations, preventive medicine (PM), and contracted vector control assets. Mosquito and sand fly populations are regional and seasonal. Other pest problems, such as bed bugs, fleas, wood infesting insects, flies, and rodents, are also relatively common in Afghanistan.

Over the past decade, entomological support to OEF has been fulfilled jointly, with the US Army and US Navy providing most of the support. Until 2010, there were generally one or 2 Army PM detachments operating in Afghanistan at any given time. In addition to the medical entomologists with the PM detachments, there was also an Army or Navy entomologist assigned to the cooperative medical assistance (CMA) unit that dealt with various medical issues among the Afghan population. US forces were heavily focused in the eastern provinces of Afghanistan. One PM detachment generally operated split-based operations between Bagram Air Field and Forward Operating Base Salerno, while another PM detachment provided support to southern Afghanistan.[1] The map shown in the Figure displays the regions and areas of responsibility across Afghanistan.

As in-theater operations intensified in late 2009 and early 2010, entomological support across the ATO increased as well. In 2009, the Navy fielded a preventive medicine detachment, modeled after an Army PM detachment, to Kandahar Air Field, providing level III PM and entomological support (described in the Table) to the southern part of Afghanistan. In early 2010, the 12th Medical Detachment moved from Kandahar to western Afghanistan to provide support to that region. The Marine Expeditionary Brigade (MEB) in Helmand Province was replaced by a larger Marine Expeditionary Force (MEF) in 2010; with one Navy entomologist supporting the Regional Command Southwest (RC-SW). The US Air Force fielded a PM detachment based on the Army model that was assigned to conduct split-based operations between Kabul and northern Afghanistan. In June 2010, the 1st Area Medical Laboratory (AML) was deployed to Afghanistan and positioned at Kandahar to provide theater-wide level IV PM and laboratory support. Along with other scientific specialists, the 1st AML deployed to Afghanistan with a medical entomologist.

As a result of the rapidly changing requirements for preventive medicine and entomological support to US forces in Afghanistan, there were 7 US military entomologists in the Afghan theater by midsummer 2010. The total included one Air Force entomologist supporting the Kabul area and northern Afghanistan; 3 Army entomologists—one supporting eastern Afghanistan, one supporting western Afghanistan, one providing theater support as part of the 1st AML; and 3 Navy entomologists—one supporting the southern region, one



The provinces, important cities, military regional commands, and NATO member country responsible for each region across Afghanistan.

supporting the southwestern region, and one working with the CMA to support counterinsurgency operations by providing entomological training to local nationals, Afghan medical personnel, and the Afghan National Army.

Prior to the autumn of 2010, the role of the theater entomologist had historically been filled as an additional duty by the CMA entomologist. This model was established because the CMA entomologist was located at Bagram Air Field along with primary headquarters elements; for several rotations the CMA entomologist was a senior entomologist; and there were very few entomologists in theater. With the changing requirements of PM and entomological support in OEF in 2010, the 62nd Medical Brigade revisited the automatic assignment of the theater entomologist duties to the CMA entomologist. Ultimately, the determination was made to select the theater medical entomologist based on a variety of factors including location in Afghanistan (with a location at one of the major airfields (Bagram or Kandahar) being preferred), rank, and previous deployment experience. As a result, the theater entomologist duties moved from the CMA entomologist in Bagram to the 1st AML entomologist at Kandahar in late 2010. When the 1st AML redeployed from Afghanistan in June 2011, the theater entomologist duties moved to the entomologist assigned to the 155th Medical Detachment at Bagram Air Field.

ENTOMOLOGICAL SUPPORT TO HELMAND PROVINCE

Entomological support to US forces in RC-SW differed from support in the rest of the regional commands since the preventive medicine support for this region did not mirror the Army preventive medicine detachment model used in the other regions. As previously described, command and control in RC-SW was transferred from a MEB to a MEF in 2010. Therefore, the preventive medicine support to the MEF was provided by the Navy and included one entomologist.

Southwestern Afghanistan, including Helmand Province, is largely a desert area with expected temperature extremes, lack of rain, and sparse vegetation. The

Helmand River, which cuts through the province, provides a significant amount of water for irrigation and other purposes throughout the region. The river valley is also a source of public health significance as the environment supports the presence of Anopheline mosquito vectors of malaria.

In 2010 and 2011, there were 2 large bases, Camps Dwyer and Leatherneck, and a number of smaller camps across the region. With much of the forces stationed at the 2 large camps, and thousands of military personnel spread among disparate forward operating bases and outposts, there was much ground to cover. The provision of preventive medicine services involved Marine and Navy operations within the constraints of a large geographic area, inadequate infrastructure, and large-scale security concerns.

Prior to 2010, the regional medical entomologist was stationed at Camp Dwyer. In 2010, after careful consideration of how to best support the region, the entomologist was moved to Camp Leatherneck, which was the logistics hub of Helmand Province, and the location of both the Defense Contract Management Activity (DCMA) and Logistics Civilian Augmentation Program activities for the region. In addition, the supervisory staff of contracted vector control was also located at Camp Leatherneck. The presence of these important groups in one location permitted the entomologist direct communication and increased influence regarding pest and vector control operations.

One significant operational consideration for Helmand Province is availability of transportation. As no formal or modern road system exists in the province, air assets are used extensively. Locating the entomologist at the air logistics hub provided the capability to travel to many areas of the Province in support of contract oversight, large-scale pest problems, or a breakout of vector-borne diseases. It was not feasible to travel around the province via convoy for regularly scheduled or reactive support. Positioning the entomologist at another forward operating base in Helmand Province would have delayed response to vector-borne diseases and pest problems.

Camp Leatherneck is also home to the largest Preventive Medicine Detachment in Helmand Province. A very important component of providing PM support to Marine forces is the provision of enlisted Navy Preventive Medicine Technicians (PMTs). Navy PMTs are broadly trained in preventive medicine, including monitoring disease and nonbattle injury, water quality/safety, food preparation and storage, and pesticide application. There are certainly limited resources, particularly available environmental health officers and entomologists, therefore, PMTs are provided to the Marines at their various echelons of command. Although PMTs are certified to apply public health pesticides and do receive baseline training on pests and vectors, they are not subject matter experts on pesticides, pests, and disease vectors. The presence of an entomologist provided synergy to PMT efforts, as they could be better directed in the reduction of pests and disease vectors. Without question, stationing an entomologist in an area where he or she can train, guide, and use PMT resources was important, not only in Helmand Province, but throughout the ATO.

The levels of preventive/environmental medicine support, compiled by the author from doctrinal publications.[2]

Preventive Medicine Support	Definition
Level I	Support provided by a field sanitation team (FST) at the company level. The FST is responsible for establishing basic sanitation measures to prevent spread of diseases.
Level II	Preventive medicine (PM) personnel at the brigade combat team level. Responsibilities include, but are not limited to, dining facility inspections, waste disposal/treatment facilities, etc; vector surveillance and control; and base camp assessments. The level II PM personnel provide direct support to the field sanitation teams within their area of operations (AO).
Level III	Support provided by PM detachments. Units are typically responsible for areas that can include multiple level II preventive medicine units. PM detachments provide support to the level I and II preventive medicine assets in their AO. Their duties include, but are not limited to, base camp assessments, epidemiological investigations, occupational and environmental health site assessments, industrial hygiene surveys, and vector surveillance and control.
Level IV	Support provided by the Area Medical Laboratory. PM responsibilities of this unit include, but are not limited to, supporting levels I – II preventive medicine as needed; testing samples for toxic industrial chemicals and materials; performing industrial hygiene surveys; performing epidemiological investigations; and performing vector testing, including pathogen detection and insecticide resistance testing.
Level V	Support provided by the US Army Public Health Command and the Navy and Marine Corps Public Health Center. Responsibilities include, but are not limited to, supporting deployed level I – IV preventive medicine personnel; performing definitive testing of air, water, and soil samples; and performing vector pathogen testing.

MAJOR ENTOMOLOGICAL ISSUES

The entomological issues that faced US forces in Afghanistan during 2010 were not unique, however, the rapidly increasing US footprint in the ATO increased the risk of vector-borne diseases and other entomological problems among US forces. Anticipating when and

where an outbreak of vector-borne disease would occur was difficult, however, efforts to improve vector surveillance and disease reporting were made in 2010.

Vector Surveillance

From the onset of operations in the Afghanistan theater until 2010, the fluid character of the operational environment and frequently shifting resource availability resulted in variations in the practice of vector surveillance across the ATO. In some areas, notably Regional Command North and Regional Command East, the level II PM personnel made efforts to conduct surveillance and submitted both sand flies and mosquitoes to the US Army Public Health Command (USAPHC) Region Europe for analysis. However, the majority of level II PM assets in theater did not conduct vector surveillance. Vector surveillance was typically performed by level III PM assets. However, in the absence of guidance as to the conduct of surveillance and what to do with insects that were collected, only about half of the level III PM units submitted specimens to USAPHC for analysis during the summer of 2010.

In an effort to ensure that vector surveillance was conducted properly and samples were submitted for laboratory analysis, a formal vector surveillance and testing program for US Forces Afghanistan (USFOR-A) was established in late spring 2011. Under this plan, the entomologists embedded with the level III PM units in each region were responsible for overseeing vector surveillance in their region and providing technical assistance as needed to level II preventive medicine units conducting vector surveillance. Sand flies, mosquitoes, and ectoparasites were submitted to the USAPHC for disease analysis, with results reported to the regional entomologists, the theater entomologist, and the clinical operations section of the medical task force for the ATO. While USAPHC (and its predecessor) had been conducting pathogen testing for vectors collected in the US Central Command (CENTCOM) area of operations for several years, formalization of the vector surveillance program in 2011 was necessary to ensure that units across theater were properly collecting and submitting vectors for testing.

Vector-borne Diseases

Cutaneous leishmaniasis (CL) remains a significant cause of disease and injury among US service members deployed to Afghanistan. In 2010 and 2011, CL was diagnosed among US forces primarily located in Regional Command South and Regional Command North. Cutaneous leishmaniasis in Afghanistan may be zoonotic cutaneous leishmaniasis, caused by *Leishmania major* (Friedlin), or anthroponotic cutaneous leishmaniasis (ACL), caused by *L. tropica* (Wright).

The majority of CL cases in 2010 and 2011 originated from Camp Mike Spann in northern Afghanistan. In this region, *L. major* is the primary cause of CL. NATO forces have experienced outbreaks in this area several times over the last decade.[3] In this epidemiological cycle, the primary vector is *Phlebotomus papatasi* (Scopoli), and the great gerbil, *Rhombomys opimus* (Lichtenstein), serves as the reservoir.[4] The preventive medicine team located at Camp Mike Spann in 2010 conducted sand fly surveillance and submitted samples to USAPHC-Europe for analysis. When the results were reported from USAPHC-Europe in early 2011, 3 of 74 pools of sand flies collected between July and September 2010 tested positive for *Leishmania* spp.

In early 2011, two cases of ACL were diagnosed in Soldiers bitten by sand flies in the Kandahar City area in the summer and fall of 2010. The Soldiers reported sleeping on the ground in open structures, not using N,N-diethyl-3-methyl-benzamide (deet) regularly, and not using bed nets. In both cases, the Soldiers had lesions for several months before seeking treatment.

Outbreaks of malaria have been reported periodically among US and coalition forces since 2001.[5,6] Malaria continued to be a problem for US forces in Afghanistan in 2010 and 2011. According to the Armed Forces Health Surveillance Center, 58 cases of malaria were reported in Afghanistan in 2010, and 91 cases in 2011.[7,8] From August through December 2010, approximately 37 cases of malaria were diagnosed in Regional Command-East, primarily from the Jalalabad area. During this period, the level II medical treatment facility located at Forward Operating Base Fenty collected blood samples from patients diagnosed with malaria at the camp. Seventeen samples were taken and both thick and thin blood smears were sent to the 1st AML for speciation. *Plasmodium vivax* (Grassi and Feletti) was the most common (14 of 17) malaria species, however, *P. falciparum* (Welch) was identified from 4 of the cases, including 2 *P. falciparum*/*P. vivax* mixed infections. Most military personnel from this region who were diagnosed reported improper use of chemoprophylaxis and/or not using appropriate personal protective measures.

In 2010 and 2011, the malaria chemoprophylaxis policy for Afghanistan was governed by USCENTCOM Individual Protection and Individual/Unit Deployment Policy,* which required all military personnel to take

*Internal, limited distribution military document not readily accessible by the general public.

prophylaxis year-round, with primaquine for postexposure prophylaxis. Since many areas of Afghanistan experience low winter temperatures, mosquitoes are not active year-round. Therefore, a concern was raised in late 2010 that the year-round prophylaxis policy was inappropriate. In order to assess the policy and examine the real risk of malaria across theater, the 62nd Medical Brigade established a malaria working group in late 2010. In the spring of 2011, the malaria working group recommended that CENTCOM examine changing the prophylaxis policy. In late 2011, the recommendation was accepted and the prophylaxis policy for Afghanistan was changed to require prophylaxis from March through November each year.

Vector Control

In 2010 and 2011, many forward operating bases and larger combat outposts in Afghanistan received vector control support from contractors. In the smaller locations without contracted vector control, the responsibility for vector control support was assumed by the entomologist assigned to the level III PM unit for the region, level II PM assets within the region, and the unit-level field sanitation teams, when present and equipped.

Locations with US Contracted Vector Control

Two companies held the contracts for pest control for US locations in the ATO in 2010, divided into 2 areas, (1) the northern and eastern regions, and (2) the southwestern and western regions. It is important to note that locations that were not under US military control had different pest control contracts which were not subject to the USFOR-A Integrated Pest Management Plan (IPMP).*

Communication was one of the major challenges for successful pest control in those locations where vector control services were provided under a US contract. As indicated in the USFOR-A IPMP, military preventive medicine assets are responsible for public health vector surveillance, whereas pest control contractors are responsible for pest surveillance and large-scale vector control. Therefore, communication between those entities is critical in preventing vector-borne diseases among US forces. Communication between the pest control managers for the 2 contracting companies and the theater entomologist has historically been good. However, communication at the local level between PM assets and pest controllers can vary widely across the theater. In many instances, there was a positive 2-way flow of information between local PM and vector control assets. At the same time, communication was poor to nonexistent in some instances, leading to delayed vector control activities and poor follow-up on the efficacy of vector surveillance efforts. In order to help mitigate this problem and ensure communication, part of the USFOR-A vector surveillance plan for 2011 included a clear directive for PM units to keep local pest control contractors informed of their surveillance results. While this requirement was previously outlined as part of the USFOR-A IPMP, mandating it as part of a fragmentary order helped ensure that the PM units were aware of this requirement.

Another major challenge for vector control contractors in the ATO was movement around the theater, especially when trying to carry pest control equipment and chemicals with them as they visited the geographically dispersed, smaller forward operating bases and combat outposts. To overcome this challenge, contractors wanted to establish pesticide storage facilities at key smaller locations to facilitate travel, increasing their ability to provide timely pest control services to those locations. Unfortunately, the language governing pesticide storage in the 2010 USFOR-A IPMP made it difficult to establish storage facilities at those locations. The 2010 USFOR-A IPMP referred to the *Armed Forces Pest Management Board Technical Guide No. 17*[9] (*TG 17*) for requirements for pesticide storage facilities. The guidance in that publication primarily addresses the storage conditions required in garrison-based operations, outside of combat zones. Unfortunately, the space and facility requirements of *TG 17* cannot always be met in contingency operations. The consensus of the theater and regional medical entomologists in the summer of 2010 was that the storage provisions outlined in *TG 17* were largely impractical for most locations in the ATO.

To address the issue of pesticide storage, the theater entomologist coordinated with other entomologists to review the pesticide storage provisions in the 2010 USFOR-A IPMP. The 2010 IPMP specifically stated:

> Permanent or semipermanent pesticide storage facilities will comply with design and construction guidance as published in the Armed Forces Pest Management Board (AFPMB) *TG 17*.

In an effort to make the requirements more appropriate for the theater and more attainable for pest control contractors, the determination was made that the entire pesticide storage section of the USFOR-A IPMP should be rewritten. The revised, 2011 IPMP included clearly defined guidelines for pesticide storage facilities that are safe and allow contractors to store a basic load of supplies at smaller locations, increasing their ability to provide timely pest control services. The 2011 IPMP still

*Internal, limited distribution military document not readily accessible by the general public.

refers to *TG 17* as a guide, but the language referring to this reference was changed considerably to state:

> all interested parties should consult the…*TG 17*…for additional guidance or ideas to improve storage facilities based on local and theater conditions.

The 2011 IPMP provided a detailed list of storage condition criteria specific for both permanent (larger bases/installations) and semipermanent (small forward operating bases and combat outposts) storage facilities to facilitate timely and effective vector control services throughout the ATO.

Locations with NATO Contract Vector Control

There are several locations throughout the ATO where US forces live on forward operating bases where contracted services, including pest control, are not provided under US contracts. At Kandahar Air Field, the largest location with a significant number of US forces, the pest control contract was managed by the NATO Maintenance and Supply Agency. The contractors are not required to comply with the USFOR-A IPMP, which outlines reporting requirements and allowable pesticides. While the contractors were not held to the USFOR-A IPMP, they had to meet reporting, pesticide use requirements, and quality control procedures outlined in their contract.

In 2010, the US population at Kandahar more than tripled. In response to this, the regional entomologist located at the Navy PM detachment worked with the contractor to foster a strong working relationship, helping to ensure that pest control met the spirit of the USFOR-A IPMP. An effort was made to ensure the insecticides in use were similar in active ingredient and concentration as those found on the AFPMB Standard Pesticides List and the DoD Contingency Pesticide List. This working relationship continued when the contractors were changed. The positive working relationship between the US Navy medical entomologist located at Kandahar in the summer of 2010 and the NATO pest control contractors was a good model for other locations in Afghanistan where similar situations existed.

ENTOMOLOGICAL SUPPORT TO STABILITY OPERATIONS

Another area where entomologists played a critical role in the ATO was their work with organizations and units supporting counterinsurgency and stability operations. The entomologist assigned to the CMA unit had such responsibilities; however, that position was discontinued in mid-2011 due to theater-wide mission changes reducing MEDCAP and VETCAP operations.* The CMA entomologist provided training on basic vector surveillance and control techniques, following the "train-the-trainer" concept, to Afghan nationals and medical personnel. The CMA entomologist also worked with agribusiness development teams and provincial reconstruction teams on crop pest management and related issues. Further, the CMA entomologist, along with regional entomologists, worked with physicians and veterinarians in regional command stability operations, the World Health Organization, the Afghan Ministry of Public Health, the National Malaria and Leishmaniasis Control Program, and various nongovernment organizations working to understand and prevent vector-borne diseases among the Afghan population. These organizations represent a wealth of knowledge and have the continuity and expertise to track and predict vector and disease outbreaks that may impact US forces. Developing and cultivating relationships with nonmilitary governmental and nongovernmental organizations working to improve public health across Afghanistan was a significant role for both the CMA and AML entomologists in 2010-2011. Despite the loss of both of those positions in 2011, efforts by the entomologists currently in theater to continue to foster these relationships would be mutually beneficial for both the Afghan population and US forces. Improving those lines of communication would continue to help reduce disease risk in the local population, and contribute to a better understanding of the vector-borne disease threat to coalition forces in different parts of the country.

SUMMARY

While the challenges and lessons learned from entomological support to the Afghanistan theater of operations in 2010 and 2011 were not novel, they provided a reminder that we often have to relearn the same lessons. The prevention of vector-borne diseases is one of the major responsibilities of deployed preventive medicine personnel at all levels. Given the wide variety of responsibilities placed on preventive medicine personnel, it can be easy to underestimate the importance of a well-designed, effective vector surveillance program. Deployed medical entomologists must champion the importance of appropriate surveillance in disease prevention to ensure that it is conducted in an effective manner. Further, entomologists must work closely with commanders to emphasize the use of personal protective measures to reduce the risk of vector-borne diseases.

Surveillance must be tied to responsive vector control efforts. In areas where surveillance is conducted by military personnel and vector control is conducted

*MEDCAP indicates medical civic action program. VETCAP indicates veterinary civic action program.

by contractors, communication between these groups is critical in ensuring that effective vector control efforts can be initiated as soon after the identification of a problem as possible. It is also important that deployed entomologists establish relationships with the appropriate DCMA personnel so that the people responsible for contract enforcement have good subject matter experts to reach out to if and when they have questions about contract compliance.

While the vector-borne disease problems in Afghanistan are not as severe as in other areas to which the US military has been and will be deployed, there is still the threat of vector-borne diseases. It is important that deployed medical entomologists maintain their focus on prevention of those diseases while balancing other mission critical tasks.

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A medical entomology class at an Afghan girls school.

A Rapid and Inexpensive Bioassay to Evaluate the Decontamination of Organophosphates

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ABSTRACT

An inexpensive and rapid bioassay using adult red flour beetles was developed for use in assessing the decontamination of environments containing organophosphates and related chemicals. A decontamination protocol was developed which demonstrated that 2 to 3 applications of 5% bleach solution were required to obtain nearly complete decontamination of malathion. The bioassay was also used to screen common household cleaners as potential decontaminating agents, but only 5% bleach was effective at improving survival of insects on steel plates treated with 25% malathion. A toxic degradation product (malaoxon) was detected using gas chromatography/mass spectrophotometry; this toxin affected the decontamination efficacy and resulted in continued toxicity to the beetles until subsequent decontaminations. The bioassay provides evidence to support the use of red flour beetles as a sensitive, less expensive method for determining safety levels of environments contaminated with malathion and other toxins, and may have application in the study of chemical warfare agents.

The organophosphates and closely related chemicals include not only important insecticides, but also potent chemical warfare agents (CWA) that have been used by militaries and terrorists alike. Important organophosphate insecticides include malathion, parathion, and chlorpyrifos which are used in a broad range of insect control programs from agriculture to public health. Among the related CWA are the neurotoxins sarin, tabun, and VX. Decontamination of areas containing these chemicals may be necessary following the inappropriate use of insecticides or following a terrorist attack with a CWA. For instance, in the early 1990s, several illegal applications of methyl-parathion, a potent agricultural insecticide, were used to control cockroach infestations in human residences in Ohio, Mississippi, Louisiana, Illinois, and Mississippi.[1] More than 4,500 homes were affected and 2 children were killed. More recently, resurging bed bug populations in the United States led to the illegal use of malathion, carbaryl, and cypermethin in over 70 houses in New Jersey, eventually requiring varying levels of decontamination.[2] Similar decontaminations have also been required after improper termiticide applications in military housing.[3,4] The threat to public health presented by inappropriately used insecticides is obvious, but the CWA are also of interest to people working in homeland security and national defense. The intentional use of sarin in the Tokyo subway system in 1995 is an example of terrorists' use of CWA. That one event resulted in 12 deaths and approximately 5,000 injuries, including injuries to first responders.[5]

Decontamination of the CWA has been defined as the "process of making any person, object or area safe by absorbing, destroying, neutralizing, making harmless, or removing chemical…agents."[6] Consistent with this definition, early attempts at CWA decontamination included washing with soap and water, absorbing with Fuller's earth, and simply leaving the chemicals to weather naturally over time. Actual chemical degradation of the toxin often relied on harsh chemicals such as calcium oxide and chlorine dioxide. New decontaminating compounds have been developed that are more effective or more environmentally friendly, including organophosphorous acid anydrolase (a hydrolyzing enzyme), and decontaminating foam with hydrogen peroxide. Much of the research required to quantify CWA decontamination requires sophisticated instrumental analytical techniques such as liquid or gas chromatography, which involves expensive equipment and trained personnel.[7-9] Extensive reviews of analytic detection and monitoring techniques are provided by Witkiewicz et al[10] and Kientz.[11] Such techniques are considered definitive but may provide

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only indirect measures of the biological toxicity. Often such processes document the breakdown of the target chemical into degradation products that are also toxic, though perhaps much less so than the original toxin. As Munnecke[12] stated, "the true change in toxicity of a pesticide containing medium can only be measured by conducting pertinent in vivo bioassays…." The same is probably true for CWA.

An inexpensive and rapid bioassay would be a useful screening tool with which to assess potential decontaminating agents for subsequent, more definitive testing by chemical analysis. Such a screening test would also be useful in quantifying or confirming changes in biological toxicity as a result of decontamination efforts. This article describes such a bioassay using an easily maintained insect colony. The bioassay is primarily intended to be a rapid screening tool. It is based on a previously published study by the first author and colleagues that demonstrated the detoxification of insecticides by certain types of paint used on ships.[13] In that study, the red flour beetle, *Tribolium castaneum* (Herbst), was used as a test organism on painted and unpainted steel plates treated with three different pesticide formulations. The mortality rate of beetles exposed to some of the insecticides was lower on painted steel plates when compared to unpainted plates. This study used a similar technique as a bioassay to investigate the level of decontamination of an organophosphate insecticide that has also been used as a CWA simulant.

MATERIALS AND METHODS

Development of Bioassay

The red flour beetle (RFB) was selected as a test organism with which to develop a rapid, inexpensive, and sensitive bioassay for evaluation of organophosphate decontamination. The RFB was used because it is easy to rear and handle in the laboratory, and has a long history of use in insecticide tests.[13] A colony of insects was obtained from the USDA Stored Products Laboratory in Manhattan, Kansas, in April 2011. The insects were raised in 946.4 ml canning jars on a diet of whole wheat flour and baker's yeast (10:1 mixture). The center of the jar lid was replaced with an unbleached coffee filter and held in place with the ring. A new colony was started every week by moving a few adults and a spoonful of flour with larvae into a new jar with the flour:yeast mixture. The jars were kept at room temperature (21°C to 24°C) in a dark cabinet with an open pan of water to provide moisture. All of the insects used for these experiments were from colonies started on April 15 or April 20, 2011.

Unpainted steel plates (20 cm by 20 cm) were cleaned with ethanol and allowed to air dry. The plates were treated with a 1-ml aliquot of 25% malathion. A commercial formulation of 50% malathion (Spectracide, Chemsico, St. Louis, MO) was used as the stock solution and was further diluted to 25% with xylene, which was also the diluent in the commercial formulation. This percentage was used because greater concentrations produced nonuniform dispersal on the plates as evidenced by oily droplets after decontamination. At 25% concentration, the malathion could be applied uniformly and it dried enough to allow the insects to move freely on the surface. The 1-ml aliquot was dripped onto the plate from a pipette, then spread evenly with a dry, 2.54 cm wide nylon bristle brush, covering the surface area of one side of the steel plate. Applications were made under a hood and the plates were allowed to dry for 48 hours. The plates then received the first decontamination.

All decontaminations were applied with an air brush (Iwata Revolution, Iwata-Medea Inc, Portland, OR) to prevent any unintended physical removal of the malathion that might occur with a brush or other application technique. The propellant for the air brush was 1,1-diflouroethane in a pressurized can. Decontaminant applications were all done in 1-ml aliquots. Potential decontaminants included:

- Standard household bleach (5% sodium hypochlorite), Clorox Bleach, The Clorox Company, Oakland, CA
- 95% ethanol
- Lysol All-Purpose Cleaner (active ingredient 3.2% lactic acid), Reckitt Benckiser Inc, Parsippany, NJ
- Simple Green Concentrate, Sunshine Makers Inc, Huntington Beach, CA
- Pine Sol Concentrate (active ingredient 8.7% pine oil), The Clorox Company, Oakland, CA

Bleach and ethanol were both selected because they are commonly used for insecticide decontamination, and bleach is a standard decontamination agent for nerve agent weapons.[9] Lysol, Simple Green, and PineSol are common household cleaners that could be used by residents of a contaminated facility to clean an insecticide-contaminated area. Ethanol and bleach were used as decontaminants in all tests, but only one of the 3 household cleaners was used in each of 3 replications. The decontamination schedule is shown in Table 1.

In each replication, the plates were decontaminated 3 times consistent with the schedule in Table 1. After each decontamination, the plates were allowed to dry

for 48 hours before the insect bioassay was performed. An untreated, nondecontaminated control was used for each replication as well as a control that was treated only with 1 ml of 5% bleach and another treated with ¾ ml of the xylene diluent. In replication No. 1, Lysol was compared to bleach and alcohol; in replication No. 2, Simple Green was substituted for Lysol, followed by Pine Sol in replication No. 3. Three to 4 plates received each treatment/decontamination combination in each of the 3 replications. A separate bioassay was run to compare the toxicity of each of these household cleaners to an untreated (no malathion) control.

For the bioassay, 10 RFB adults were counted into small disposable Petri dishes and allowed to starve overnight. They were observed the next day to ensure that they were still alive, then one side of the Petri dish with the RFB inside was inverted on the steel plates, exposing the insects to the treated surfaces. After one hour, the plates were inverted, collecting the RFB back into the Petri dish and the insects were observed for toxic effects. All of the insects in each dish were immediately observed and placed into one of the following 3 categories:

Category 1: Alive (moves when prodded with a probe).

Category 2: Knockdown (moribund but showing some movement of legs or head)

Category 3: Dead (total lack of movement even when prodded with a probe)

The lids were replaced on the Petri dishes and the insects were allowed to sit undisturbed on the bench top until they were evaluated again for toxic effects 24-hours postexposure. This process was performed after each of the 3 decontaminations. At the end of each bioassay, the tested insects were destroyed and not used for subsequent bioassays.

Statistical Analysis

Data were analyzed using the PROC ANOVA procedure in the SAS 9.2 software (SAS Institute Inc, Cary, NC). The models were used to describe the effects of decontamination on survival (those classified as alive) at 1-hour postexposure and at 24-hours postexposure. Another category of some movement combined the categories of alive and knockdown. Means of survival and survival with knockdown were compared using the Tukey's HSD (honest significant difference) test.

Table 1. Application and bioassay schedule for decontamination of malathion with common household cleaners.

Day 0	25% malathion applied to plates
Day 2	First decontamination
Day 4	First bioassay; second decontamination of plates
Day 6	Second bioassay; third decontamination of plates
Day 8	Third bioassay

Comparison to Standard Chemical Assay

The described steel plate assay was used to compare to a standard analytical process using gas chromatography/mass spectrophotometry (GC/MS). Twelve steel plates were set up as in the assay as previously explained, and then smaller steel plates (5.08 cm by 5.08 cm) were placed in the center of each plate as coupons. The coupons were taped onto the larger steel plates with only a small edge of the laboratory tape extending onto the coupon. This was done to prevent treatments from contaminating the underside of the coupon. The plates and coupons were then treated with 1-ml aliquots of 25% malathion and dried for 24 hours. Four of the coupons were then removed, placed in glass jars, and transported to the chemistry laboratory at the Jordan Valley Innovation Center, Missouri State University, for analysis. At that time, the remaining plates with coupons received a single decontamination with 5% bleach identical to that described earlier. After 24 hours, 4 more coupons were removed for chemical analysis, and the remaining plates with coupons received a second decontamination treatment. Following another 24 hours, two of the coupons were removed for analysis and the last two plates with coupons received a third decontamination treatment. After another 24 hours, the bioassay was performed on the larger steel plates, placing the Petri dishes on treated (malathion) and decontaminated (bleach) areas next to the sites where the coupons had been removed.

Reagents and Materials. Malathion and malaoxon PESTANAL analytical standards were purchased from Sigma-Aldrich (St Louis, MO). Optima grade acetone was purchased from Fisher Scientific (Fair Lawn, NJ). Stock standards of 150 µg/ml and 100 µg/ml were freshly prepared in acetone each week and stored at 4ºC in opaque Nalgene bottles (Fisher Scientific). Calibration standards (0.1 µg/ml - 150 µg/ml) were also prepared weekly in acetone from the stock standards by serial dilution in acetone and stored at 4ºC in opaque Nalgene bottles.

Sample Extraction. The sample extraction procedure was adapted from Rogers et al.[14] The coupons were removed from the treated steel plates and placed in 250 ml glass straight-sided jars (Fisher Scientific) to which 80 ml of acetone was added. The samples were then sonicated for 30 minutes. After sonication, 1 ml was removed from the jar and added to an autosampler vial for GC/MS analysis. A single extraction cycle proved to be sufficient for the steel coupons.

GC/MS Analysis. A Varian 450 GC (Varian Medical Systems Inc, Palo Alto, CA), coupled to a Varian 320 triple quadropole mass spectrometer was used for the analysis. The instruments were interfaced to a computer running Varian MS workstation version 6.9.1 for instrument control and data processing. Instrument conditions were similar to those used in Rogers et al.[14] The column used for separation was a Zebon ZB-1701 with 5 mm Guardian guard column (Phenomenex, Torrance, CA). The column dimensions were 30 mm by 0.25 mm by 0.15 µm film thickness. A 1 µl sample was injected in splitless mode at 200ºC. The GC oven was programmed as follows: 100ºC hold for 2 minutes, increased to 180ºC at a rate of 10ºC/minute, increased to 220ºC at a rate of 5ºC/minute, increased to 260ºC at a rate of 20ºC/minute and held at 260ºC for 2 minutes. Total run time was 22 minutes. Helium was the carrier gas with a flow rate of 0.8 ml/minute. The mass spectrometer was operated in electron impact mode. The transfer line and ion source temperatures were set to 280ºC and 230ºC, respectively. Retention times were determined and parent ions were verified in full scan mode. Quantification and qualification ions were selected and collision energies were determined experimentally by tandem mass spectrometry. Analyte specific information is shown in Table 2.

Peak areas of standards were plotted using a quadratic function with weighting scaled by the inverse of analyte concentration. A minimum of 6 points was required for an acceptable calibration curve. Both calibration curves had correlation coefficients of $r^2 > 0.990$.

Statistical Analysis. Mean concentrations were calculated with the PROC MEANS procedure in SAS 9.2 for both malathion and malaoxon on the coupons. Results were graphed using a Microsoft Excel 2010 spreadsheet.

RESULTS

Bioassay Development

Survival on plates that did not receive an application of malathion or a decontaminant was not different from plates that received only a xylene application (0.75 ml) or 1 to 3 applications of 5% bleach ($r^2 = 0.01$, $P = .65$). This finding indicated negligible toxicity of the diluent (xylene) and the standard decontaminant (5% bleach). Due to the lack of toxicity on these plates, the control throughout the study was subsequently defined as plates that did not receive a malathion application or any decontamination treatment, or that received only a bleach or xylene application. Similarly, survival on plates that were treated only with the decontamination agents of Lysol, Pine Sol, Simple Green, and ethanol was not significantly different from an untreated (no malathion) and undecontaminated control.

Survival levels on plates treated with malathion but which were not decontaminated were consistent throughout the multiday study for each replication. The last bioassay on each replication was run 8 days after the initial application of malathion, but survival levels on control plates on the last bioassay was not different from that of the first or second bioassays in each replication ($r^2 = 0.002$, $P = .97$). This finding provides evidence that in the protected environment of the laboratory, malathion was not degraded and it remained active throughout the duration of the biological testing.

Table 3 displays the survival of 10 RFB confined for one hour on steel plates treated with malathion, then decontaminated with 1, 2, or 3 treatments of 5% bleach solution. Bleach was used as the standard decontamination treatment for this study. To monitor changes in the level of toxicity to each treatment, survival levels were measured one hour and 24 hours after initial exposure, and as a combined measurement of survival and knockdown (some movement) 24 hours after initial exposure. A means separation test indicated that the first application of a decontaminant on Day 2 of the experiment did not result in significant detoxification. This finding was consistent with all 3 measures of toxicity. However, after a second application of the bleach solution on Day 4, survival was significantly increased on the plates as measured by the bioassay on Day 6. This finding was also consistent with all 3 measures of toxicity. After a third decontamination of the plates on Day 6, the survival level as measured on Day 8 was slightly greater, but was not significantly different from that of the second decontamination. Survival levels after the second and third decontaminations were not significantly different from that on plates that did not receive an application of malathion except as measured by simple survival (not knockdown) at 24 hours. That measure indicated a difference in survival on malathion-treated plates that received only 2 decontaminations as compared to plates that received no malathion but did receive bleach applications. Of the 3 measures of toxicity, the measure of "some movement" explained the greatest

Table 2. Analyte information for gas chromatography/mass spectrophotometry analysis.

Analyte	MW	Parent Ion	Retention time (min)	Quantification Ion (m/z)	Qualification Ion (m/z)	Collision Energy
Malathion	330.36	331	17.2	285	173	5 eV
Malaoxon	314.30	315	17.7	173	127	6 eV

MW indicates molecular weight.
m/z indicates mass to charge ratio.

Table 3. Postexposure percentage of surviving *Tribolium castaneaum* after confinement for one hour on 20 cm by 20 cm steel plates which had been treated with 1 ml of 25% malathion, then decontaminated sequentially with 1, 2, or 3 applications (1 ml) of 5% bleach solution.

Number of decontamination treatments	n	Survival 1-hour postexposure % (SD)	Survival 24-hours postexposure % (SD)	Some movement* 24-hours postexposure % (SD)
0	36	59.7 (26.4)a	30.8 (22.9)a	30.8 (22.9)a
1	12	44.2 (28.1)a	12.5 (31.0)a	24.2 (35.0)a
2	12	82.0 (28.0)b	55.0 (40.5)b	85.8 (18.3)b
3	12	96.7 (4.5)b	80.0 (34.9)b,c	97.5 (4.5)b
Bleach only	27	97.7 (4.4)b	98.8 (3.3)c	98.8 (3.3)b
r^2		0.40	0.62	0.70

n indicates the number of steel plates that received the designated treatment.

Note: Values in a column followed by the same letter are not significantly different (Tukey test, *P*=.05).

*Combined category including insects that were alive or knocked down.

amount of variation in the model as demonstrated by an r^2 value of 0.70.

None of the household cleaners appeared to decrease toxicity, with only bleach demonstrating a decontaminating effect as demonstrated by the bioassay. Table 4 compares the standard (5% bleach) to common household and laboratory cleaners in their capacities to decontaminate malathion. The measures of toxicity were the same as used earlier. All treatments for each decontaminant were combined for comparison in Table 4. There was decreased survival on plates treated with Pine Sol and Simple Green alone as compared to the nondecontaminated control, a finding that is inconsistent with the control studies that indicated no toxicity due to the decontaminating agents alone. Survival on bleach-treated plates was significantly greater than on plates decontaminated with any of the other agents.

GC/MS assay

To correlate data from the bioassay experiments to the degradation of malathion, GC/MS was used to measure the amount of malathion and malaoxon, the oxidative byproduct of the decontamination of malathion. Because malaoxon is toxic, the concentration of this chemical was also determined in the GC/MS assay. Concentrations of malathion and malaoxon after 0 to 3 decontaminations are graphically depicted in the Figure. The presence of malaoxon as a toxic byproduct of the oxidation of malathion continued after 1 and 2 applications of bleach decontaminant, but was completely removed after a third application Three decontaminations resulted in almost complete degradation of malathion.

COMMENT

The simple bioassay demonstrated in this study provides a quick screening mechanism that can be used to investigate factors affecting the decontamination of neurotoxic chemicals, particularly the organophosphates. It allowed the identification of an effective application rate of bleach for use as a decontaminating agent. This is perhaps the greatest utility of the bioassay. When the toxic agent was 25% malathion, about twice as much 5% bleach by volume was required to significantly improve survival of RFB on malathion treated plates. Nearly complete decontamination was obtained by 3 subsequent applications of bleach with each application being the same size by volume as the 25% malathion. This rapid assessment of efficacy can be useful when putting together decontamination protocols for toxic agents, especially because it measures actual biological toxicity. Further research is necessary to determine if lower concentrations of the bleach decontaminant or smaller aliquots might be effective in repeated decontaminations. The concentration used in this study (5%) was very high and would not be suitable for use in many situations.

Table 4. Postexposure percentage of surviving *Tribolium castaneaum* after confinement for one hour on 20 cm by 20 cm steel plates which had been treated with 1 ml of 25% malathion, then decontaminated sequentially with a common household cleaning product.

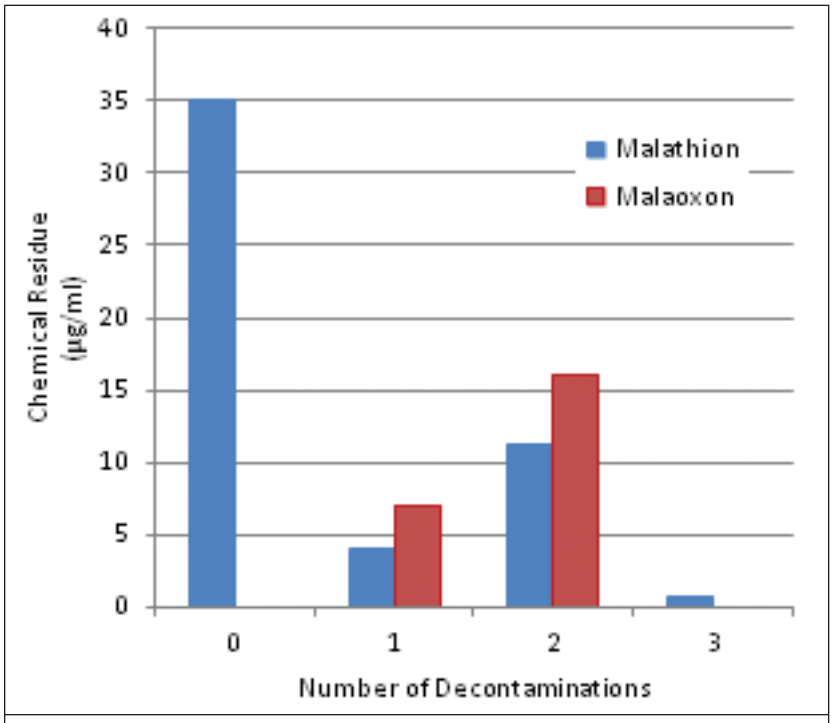
Decontaminant	n	Survival 1-hour postexposure % (SD)	Survival 24-hours postexposure % (SD)	Some movement* 24-hours postexposure % (SD)
No decontamination	98	8.1 (20)a	9.5 (20.0)a	30.8 (22.0)b
Pine solvent	9	3.3 (5.0)a	1.1 (3.3)a	3.3 (5.0)a
Simple Green	12	8.3 (11.0)a	1.7 (3.9)a	15.0 (23.5)a,b
Ethanol	23	3.9 (6.6)a	16.0 (25.0)a	37.8 (32.0)b
Lysol	12	18.3 (25.1)a	20.0 (26.9)a	41.7 (31.6)b
Bleach (5%)	24	61.2 (38.4)b	67.0 (39.0)b	91.7 (14.3)c
No malathion/no decontamination	37	96.6 (7.7)b	95.2 (12.0)b	98.6 (3.5)c
r^2		0.85	0.80	0.79

n indicates the number of steel plates that received the designated treatment.

Note: Values in a column followed by the same letter are not significantly different (Tukey test, *P*=.05).

*Combined category including insects that were alive or knocked down.

Chemical analysis of the residual toxin on decontaminated plates confirmed that a significant amount of malathion remains on the plates after one decontamination; the concentration of degradation byproducts was also increased. When 25% malathion was decontaminated with 5% bleach, nearly complete decontamination of the surface was achieved with 3 treatments, a finding consistent with both the bioassay and the GC/MS analysis. The presence of a toxic byproduct of decontamination (malaoxon) was demonstrated by both the chemical analysis and suggested by the bioassay, demonstrating the need for validated protocols for decontamination processes.



Mean malathion and malaoxon residuals (µg/ml) on steel plates after 0, 1, 2, or 3 applications of 5% sodium hypochlorite (bleach) solution applied with an air brush.

A rapid screening of household cleaners using the RFB bioassay failed to identify any additional decontaminating agent other than the common bleach solution already known to be an effective decontaminant. Other cleaners like Simple Green and Pine Sol might be useful in the physical removal of the agent, but do not demonstrate a reduction in toxicity of malathion as determined by the insect bioassay. The results of the bioassay, however, were not always straightforward. The increased toxicity on malathion-treated plates decontaminated with Simple Green and Pine Sol was unexpected. These undiluted substances are slightly viscous. Perhaps this physical characteristic impedes the insect's movement or covers the spiracles leading to asphyxiation. Alternatively, the cleaners may break down protective characteristics of the insect cuticle, thereby increasing the insect's susceptibility to the toxin. Insects exposed to plates that received only Pine Sol or Simple Green applications, but no malathion, did not elicit greater mortality than did plates that had received no application. This suggests that these 2 cleaners may somehow synergize the action of the malathion, though this possibility would require more research to confirm. Another interesting finding in this screening was the lack of efficacy of ethanol as a decontaminating agent against malathion. Ethanol has been used as a decontaminant for other insecticides, specifically organochlorines,[4] but did not show any efficacy against an organophosphate.

The benefits of this bioassay include its rapidity, very low expense, and its actual measurement of biological toxicity. The latter is important given that analytical chemistry-based measures can quantify the breakdown of the target chemical, but may fail to measure the toxicity of degradation products. No expensive equipment is required and this bioassay could actually be performed in a field situation with only minor modifications. This type of bioassay provides almost immediate results and can easily be adapted to test a variety of surfaces such as concrete, wood, and tile. It can also be used to study the impact of environmental variables such as temperature, humidity, and insolation on the decontamination of toxic chemicals. However, this bioassay does not identify the mode of toxic action, nor does it rule out the possibility of other forms of toxicity such as endocrine disruption or carcinogenicity. Since this bioassay does not identify the mechanism by which the insects are killed, it is not a replacement for the standard analyses involving analytical chemistry. Also, the surfaces to be tested must be dry. Wet surfaces lead to concentration of the toxins or decontaminants and wetting of the insect cuticle, both of which can cause inconsistent measures of toxicity. This phenomenon was particularly observable with the Lysol applications and may limit the utility of the bioassay for such decontaminants.

Future research using this bioassay will include investigations of decontamination efficacy on various surface types, extended screening of potential decontamination agents, and evaluation of environmental factors such as temperature and humidity on decontamination processes. Although the current screening was done with

decontamination of an insecticide on steel plates as a model, this bioassay may also serve as a method to study the decontamination of a variety of toxic environments such as facilities that have been contaminated during inappropriate termiticide applications, chemical warfare agent attacks by terrorists or national militaries, or even houses contaminated by the illegal manufacture of methamphetamines. It would be most useful when used as an initial screening tool as it is not a replacement for the more comprehensive and expensive analytical tests.

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Review of the Institute of Medicine Report: Long-term Health Consequences of Exposure to Burn Pits in Iraq and Afghanistan

Coleen P. Baird, MD, MPH

BACKGROUND

A previous issue of the *AMEDD Journal* included an article[1] I wrote concerning solid waste disposal in the US Central Command (CENTCOM) area of operations. That article described how the Department of Defense (DoD) conducted numerous monitoring studies at Joint Base Balad (JBB), the location with the US military's largest burn pit in theater. Screening health risk assessments, publicly released in 2008,[2] stated that the burn pits at JBB and other US military locations in Iraq posed an "acceptable health risk" based on the contaminant levels measured. While sampling can be used to identify a potential concern, it does not refute every concern because it cannot address all locations and conditions at all times. I discussed the limitations of the sampling efforts, including the points that sampling identifies conditions at the time of sampling, and that burn pit sampling was intermittent while waste streams and meteorological conditions were variable. As waste streams vary, analyte concentrations would be expected to vary. The methodology does not incorporate particulate matter (PM) such as PM_{10} or $PM_{2.5}$ concentrations. Particulate matter is a mixture, and does not have a toxicological value for use in the methodology. The list of analytes was not exhaustive. Screening health risk assessments indicate the general probability that a risk is present under very specific exposure conditions. This can be useful to make decisions regarding the need to take remedial actions, but is not well-suited to inform regarding an individual's health risk.

To further evaluate the potential health impact of burning trash, the Armed Forces Health Surveillance Center conducted a retrospective cohort study[3] to compare the incidence rates among deployers and nondeployers for respiratory diseases, circulatory diseases, cardiovascular disease, ill-defined conditions, and sleep apnea; compare the responses on the postdeployment health assessment forms among individuals deployed to one of several CENTCOM locations with and without burn pits; and compare the rates and proportions of medical encounters for respiratory outcomes while assigned to various CENTCOM locations. To address the issue of particulate matter exposure, service members in Korea (where particulate matter levels are high) were also included, as well as a nondeployed control population. The main finding was that for nearly all health outcomes measured, service members from the CENTCOM locations and Korea had either similar or significantly lower incidence rates compared to the US-based cohort. The exception was that personnel assigned to a deployed site without a burn pit had a measurably higher rate of signs, symptoms, and ill-defined conditions noted postdeployment. For health outcomes measured during the deployment period, Air Force personnel at Joint Base Balad had a higher proportion of respiratory encounters, yet this was not noted among Army members at the same location, or for military personnel at the other burn pit sites which were studied. The report concluded that, while the study had limitations, the results taken collectively generally showed no impact of burn pit exposure several years postdeployment. They recommended further improvement in the quality of individual-level exposure data, to include data from additional burn pit sites, and further investigation of possible long-term health effects. Strengths of the study included the ability to use comprehensive electronic medical records and the large size of the population for statistical analysis. As with many epidemiological studies, limitations are also recognized, for example, measures of individual exposures, some exposure misclassification, lack of information on job duties, and information on smoking behavior.

Personnel are exposed to dust and ambient particulate matter while deployed, often in conjunction with other exposures such as burn pit smoke and local industrial emissions. Sampling data indicates variable conditions to include occasions where levels exceed certain health guidelines.[4] High levels of ambient particulate matter and burn pit smoke can irritate the eyes and respiratory passages at the time of exposure. Air pollution literature indicates that such exposures could cause or exacerbate chronic lung conditions, including chronic bronchitis and asthma, with effects dependant on the degree and duration of exposure, as well as characteristics of the population being exposed.[5,6] The completed scientific

studies evaluating the association between environmental exposures encountered during deployment to Iraq and/or Afghanistan and lung health indicate a range of different findings. These include no evidence of an association between deployment-related exposures and chronic respiratory conditions,[3] an association between specific lung diseases and deployment,[7] and evidence of increased respiratory symptoms but not a specific diagnosed disease.[8] Additional conditions such as acute eosinophilic pneumonia and constrictive bronchiolitis are described in case series from which epidemiologic associations cannot be directly estimated.[9,10] Although all of these studies have methodological limitations that constrain the strength of the conclusions being drawn, their findings warrant continued investigation.

Given the lack of clear consensus and in response to concerns expressed by military personnel, Veterans, their families, and Congress, the Department of Veterans Affairs (VA) asked the Institute of Medicine (IOM)* to:

> Determine the long-term health effects from exposure to burn pits in Iraq and Afghanistan. Specifically, the committee will use the Balad Burn Pit in Iraq as an example and examine existing literature that has detailed the types of substances burned in the pits and their by-products.[11(p1)]

APPROACH USED BY THE IOM COMMITTEE

The committee used 3 sources of information in their deliberations. The first was the actual monitoring data from JBB. The report noted that while the Balad assessments were useful, information regarding the waste streams at particular locations was not available apart from general information on percentages of plastics, wood, metal, and other combustible and noncombustible items burned. They also acknowledged that the sampling did not include criteria pollutants such as ozone, carbon monoxide, and sulfur dioxide, although this was due to difficulties in conducting this sampling in a deployed setting. They concluded that background ambient air concentrations of PM were high, with average concentrations above US air pollution standards, and were most likely derived from local sources. Dioxin compounds were detected at low concentrations, although high even when compared to polluted urban areas, and the burn pit was the likely source. Volatile organic compounds and polycyclic aromatic hydrocarbons were similar to those reported for polluted urban environments outside the United States. The report concluded that personnel were exposed to a mixture of combustion products from the burn pit and other air pollutants from local and regional sources, including other combustion sources, ground transportation, stationary power generation, the Balad airport, other industry, and wind-blown soil.

The second phase of their approach involved a review of the monitoring data from JBB and evaluation of the potential health effects of compounds detected in more than 5% of samples, or expected to be present. There were 51 such compounds which were evaluated for potential cancer and noncancer health effects. These chemicals were categorized as dioxins and furans, volatile organic compounds, and particulate matter. The "potential health effects" associated with exposure to these chemicals at sufficient levels were stated as:

- Neurological effects and reduced central nervous system functions
- Liver toxicity and reduced liver function
- Cancer (stomach, respiratory, skin, leukemia, others)
- Respiratory toxicity and morbidity
- Kidney toxicity and reduced kidney function
- Blood effects (anemia, etc)
- Cardiovascular toxicity and morbidity
- Reproductive and developmental toxicity

The committee acknowledged that potential health effects associated with any single compound have "little predictive value" for deployed personnel at JBB. This determination was reached because although most of the detected pollutants were present at concentrations lower than the health-based reference values, sampling was limited in time. Individual exposure levels would be expected to vary. For example, exposure would be higher for those who worked at or near the burn pit (which in fact was quite large) than for those located elsewhere on the Balad camp. Additionally, concentration of PM exceeded US standards, but PM composition and characteristics can vary with the source and contributors. Literature on the health effects of exposure to mixtures, and specifically mixtures from burn pits is minimal.

These issues led the committee to the third aspect of their assessment; a review of the epidemiologic literature on health outcomes associated with exposure to burn pit emissions (military studies) and other populations with exposure to similar combustion sources (firefighters, workers at municipal incinerators, residents who live near incinerators, and Veterans of the 1990-1991 Persian Gulf War who were exposed to smoke from oil-well

*The Institute of Medicine is the health component of the National Academy of Sciences. It is an independent, nonprofit organization that works outside of government to provide unbiased and authoritative advice to decision makers and the public. Information: http://www.iom.edu/About-IOM.aspx.

fires) to identify health outcomes potentially related to combustion products similar to burn pit emissions. All studies are known to have limitations and uncertainties, including the "healthy worker" effect, exposure misclassification, lack of information on confounders, inadequate statistical power, disease misclassification and publication bias. Considering these and the information from the studies reviewed, the committee then adopted the categories of association used for previous reports regarding the first Gulf War and health, and Agent Orange and health, based on the weight of evidence. The 5 categories were sufficient evidence of a causal relationship, sufficient evidence of an association, and limited, suggestive, or inadequate evidence based on available data.

FINDINGS OF THE COMMITTEE

As noted, the report concluded that the mixture of chemicals from regional background and local sources that contribute to the high PM may be of the greatest concern at JBB. As previously described, the PM consists of windblown dusts and elemental carbon and metals that arise from transportation and local industrial activities.[12] The committee considers the air pollution literature related to particulate matter to be relevant to military personnel deployed to the Middle East. Weese and Abraham[4] noted that the potential health implications of the PM measured in an extensive sampling event in deployed locations in the Middle East included respiratory and cardiovascular outcomes.

Overall, the committee concluded:

> In light of its assessment of health effects that may result from exposure to air pollutants detected at JBB and its review of the literature on long-term health effects in surrogate populations, the committee is unable to say whether long-term health effects are likely to result from exposure to emissions from the burn pit at JBB. However, the committee's review of the literature and the data from JBB suggests that service in Iraq and Afghanistan—that is, a broader consideration of air pollution that exposure only to burn pit emissions—might be associated with long-term health effects, particularly in highly exposed populations (such as those worked at the burn pit, or susceptible populations (for example, those who have asthma) mainly because of the high ambient concentrations of PM from both natural and anthropogenic, including military, sources. If that broader exposure to air pollution turns out to be sufficiently high, potentially related health effects of concern are respiratory and cardiovascular effects and cancer.[11(p7)]

The committee determined that there is inadequate/insufficient evidence of an association between exposure to combustion products and cancer, respiratory disease, circulatory disease, neurologic disease, and adverse reproductive and developmental outcomes in the population studied. The report concluded that there was limited/suggestive evidence of an association between exposure to combustion products and reduced pulmonary function in the populations studied. It was acknowledged that the results in the populations studied (firefighters and people living near incinerators) may not be generally applicable to military personnel exposed to emissions from burn pits.

So what does this really mean? The report noted that no individual chemical constituent of the combustion products emitted at JBB was measured at levels likely to be responsible for adverse health effects discussed. However, the sampling had limitations and the issue of mixed and cumulative exposures remains. These include not only PM and combustion products, but other interacting factors such as stress, smoking, and local climatic conditions. The report identifies the value of better exposure characterization. Individual monitoring data would greatly reduce misclassification bias and potentially allow for an assessment of dose/response, but the burn pit in Balad has closed and the drawdown in troops in the CENTCOM area makes extensive monitoring unlikely. Also recommended was a prospective study on individuals who were deployed to Balad, with an attempt to classify the exposure into low, medium, and high. Identifying highly exposed subgroups within those deployed to Balad is not possible from deployment location records, but comparison of groups deployed there during the height of burn pit use as opposed to after initiation of incinerator use is another approach. Additionally, it recommended extending the study for a longer time period to address conditions which would not arise immediately due to latency. This is being accomplished. The Armed Forces Health Surveillance Center is repeating the prior study with a longer period of follow-up. This study design was very similar to the design recommended by the Institute of Medicine, including outcomes associated with proximity to exposure and outcome comparisons between deployed personnel who were exposed to burn pits and deployed personnel without such exposure. An independent oversight committee comprised of military and external experts was also recommended, and is being explored.

It was noted that pilot studies should be conducted to address the issues of statistical power and to develop design features for specific health outcomes. At present, studies are being conducted among a predeployment population at Fort Hood, Texas, and in a new recruit population at Fort Sam Houston, Texas, to assess the feasibility of conducting baseline spirometry at predeployment and at accession into the military. The use of

screening spirometry in an asymptomatic population is not currently recommended.[13,14] However, the rational for baseline testing is that military members, as a group, might have better lung function than the reference populations used for comparison. Traditional evaluation of pulmonary function determines whether test results are in the normal range, which is based on asymptomatic nonsmokers. If individuals have above average lung function at baseline, it is possible over time to drop, unnoticed, from the top to the bottom of the normal range without dropping below the normal range. In this case, changes from a baseline would be more meaningful and might detect impacts to pulmonary function earlier.[15]

It is also important to obtain smoking status for use in epidemiological studies, and to move towards a smoke-free Army. A prospective evaluation performed by the Millennium Cohort Study team* found greater percentages of smoking initiation in never-smokers, smoking resumption in past smokers, and increased smoking in current smokers among service members with a history of deployment when compared to nondeployers.[17] Smoking is typically raised as an issue when discussing respiratory health outcomes, but not merely to attribute health effects to another cause. The inflammation associated particularly with new-onset smoking might make one more susceptible to exposures. For example, acute eosinophilic pneumonia, a serious but uncommon respiratory condition diagnosed in some deployed individuals, appears to be related to new-onset smoking.[9]

The DoD and VA have recognized the need to address burn pit exposure as well as other exposure effects on pulmonary function and disease. Additionally, organ systems other than the pulmonary system are affected by at least some of the chemicals involved with burn pit smoke and other airborne exposures. The DoD and VA now consider deployment-related airborne hazards a better focus than burn pits. The first study by the Armed Forces Health Surveillance Center used locations in the CENTCOM area of operations (specifically, 2 burn pit sites and 2 nonburn pit sites). The selected locations allowed comparisons between sites where individuals were exposed to burn pit smoke and sites where individuals were not exposed to burn pit smoke, but were exposed to other pollution sources, such as particulate matter and pollution from local industry. The studies evaluated circulatory diseases, cardiovascular disease, signs, symptoms, and ill-defined conditions, as well as respiratory diseases. The Naval Health Research Center component of the study evaluated birth outcomes in infants whose mothers and fathers had been exposed before and during pregnancy, chronic multisystem illness, lupus, and rheumatoid arthritis. Continued follow-up of these cohorts will provide information regarding deployment related exposures in addition to burn pits and any health condition can be included in the analysis.

A number of Veterans groups have worked for recently introduced federal legislation that mandates a VA burn pit registry.[18] According to Congressman Todd Akin, who authored the legislation:

> Unfortunately the VA has struggled to help these Veterans. Creating a burn pit registry is an important step to help these Veterans get the care and support they need and that our nation has promised them.[18]

However, given that the IOM concluded that ambient air pollution may pose greater health risks than chemicals emitted from military burn pits, mandating a registry of those exposed to burn pits might not target individuals at risk of health outcomes. Registries enable medical follow-up and outreach efforts. A registry and subsequent studies limited to those who self-report exposure to burn pits may miss identifying adverse health effects in those exposed to wider pollution. According to the IOM report, there are no illnesses specifically associated with burn pit exposure and, therefore, no markers to allow for medical follow-up. However, the broader question of deployment-related inhalational exposures and health outcomes deserves continued study.

*The Millennium Cohort Study is an ongoing, longitudinal cohort study designed to evaluate any long-term health effects of military service, including deployments. Based at the Naval Health Research Center in San Diego, CA, it is the largest prospective study ever undertaken in the US military.[16]

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Hospitalization and Medical Evacuation of Army Personnel Due to Toxic Inhalational Exposure—Operations Iraqi Freedom and Enduring Freedom, 2001 Through Mid 2011

Jessica M. Sharkey, MPH

BACKGROUND

Problem Statement

Following the first Gulf War (1991), concerns related to potential toxic inhalational exposures among military personnel were raised that remain unresolved. Similar exposure concerns during deployment have arisen as a result of current efforts, including military support of Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF). During these conflicts, extensive intermittent ambient sampling has been conducted at select locations within the US Central Command area of operations (CENTCOM AO)[1] and questionnaires aimed at documenting service members' environmental exposures while deployed have been completed. Some work has been done to characterize exposure to specific exposure events on a population level. However, research regarding the possible relationship(s) between deployment-associated toxic inhalational exposures and subsequent health outcomes is limited, due to the fact that quantitative, verifiable exposure information at the individual level is difficult to ascertain.

Potential Exposures

The current conflicts in Southwest Asia comprise the longest period of continuous armed conflict in US history. As a result, almost all US military personnel serving since 2001 have deployed in support of these operations,[2,3] typically 12 months at a time, with many service members deploying multiple times. As a result, concerns regarding environmental exposures during deployment have been documented frequently by deployed service members.[4] Helmer et al found that concern for poor air quality from burning trash, smoke from oil well fires, and sand/dust was documented in 34%, 20%, and 16%, respectively, of reviewed medical records.[5] Air sampling conducted by the Department of Defense (DoD) in Southwest Asia areas of operation identified particulate matter (PM) as a major environmental exposure of concern.[6] Deployment-associated sources of PM include fine sand and dust that was resuspended by strong winds or troop activities, exhaust from the engines of heavy machinery (gas, diesel, turbine), the industrial landscape that is characteristic of some locales, and potentially toxic smoke from burn pits and fires.[6] Concern regarding these population level deployment-related exposures, as well as uncertainty regarding the health effect of combined exposures has highlighted the need for deeper understanding of individual environmental exposure during deployment. Gaining this understanding is complicated by the fact that personal exposure experiences vary with the unit, their mission, and location. Further, there may be unplanned or accidental exposures not reflected in available aggregate sampling data.

Exposure Events

Large populations have potentially had inhalational exposures due to ambient conditions, localized sources (industry), unplanned events (releases/venting of toxic substances, fires), and activities such as waste management. Available sampling data indicate that measured levels are rarely of acute concern, but if sustained, may be associated with or contribute to chronic health effects. The availability of sampling data varies, with most available data reflecting ambient conditions, and minimal data available for unplanned incidents.

Burn Pits

In addition to naturally occurring, ambient PM, burn pits are being recognized as a contributing source of PM at many OIF and OEF deployment locations in Southwest Asia.[4,7] Prior to 2010, burn pits were widely used as waste management tools at locations where more sophisticated methods of solid waste disposal (incinerators, reuse/recycling, containerized removal by contractors) were not feasible methods for trash disposal in the wartime environment. A burn pit is formally defined as:

> an area, not containing…an incinerator or other equipment specifically designed…for burning of solid waste, designated for the purpose of disposing of solid waste by burning in the outdoor air at a location with more than 100 attached or assigned personnel and that is in place longer than 90 days.[8]

Open-air burn pit use is now minimized due to the potential for both short-term and long-term health effects.

In response to concerns regarding the possible health effects brought about or exacerbated by exposure to smoke plumes originating from burn pits during deployment, several research initiatives to examine potential associations have been conducted. The Armed Forces Health Surveillance Center, Naval Health Research Center, and US Army Public Health Command conducted a series of evaluations that found exposure to burn pits was not associated with incidents of or worsening prevalent respiratory health diseases. However, it was acknowledged that, in light of study limitations, further investigation is needed to establish a better understanding of the relationship between exposure and outcome.[9] The Institute of Medicine* recently released the results of their study, *Long-Term Health Consequences of Exposure to Burn Pits in Iraq and Afghanistan*†,[7] which, among other things, concluded that it is uncertain whether exposures to emissions from burn pits have caused long-term health effects. But the report also included:

> …the committee's review of the literature and the data from JBB [Joint Base Balad] suggests that service in Iraq or Afghanistan—that is, a broader consideration of air pollution than exposure only to burn pit emissions—might be associated with long-term health effects, particularly in susceptible (for example, those who have asthma) or highly exposed subpopulations (such as those who worked at the burn pit).[7(p114)]

Despite the current absence of definitive evidence citing a direct link between burn pit smoke exposure and long-term chronic respiratory conditions among OIF and OEF veterans, deployed military forces, following DoD policies, have been decreasing their reliance on burn pits.

Fires

Not surprisingly, significant fire events have been documented in the combat theaters of Southwest Asia. The impact on the environment included air pollutants that may have had potentially detrimental consequences on the respiratory health of exposed military personnel. One study conducted after the Kuwait oil well fires at the end of the 1991 Gulf War found a statistically significant positive association between the prevalence of self-reported exposure to the smoke and self-reported symptoms of asthma and bronchitis. That association did not hold when modeled exposure was assessed in conjunction with the same respiratory outcomes.[10] Petruccelli et al found increased reports of respiratory symptoms (such as cough, shortness of breath, upper respiratory tract irritation) among Soldiers with potential exposure to the Kuwait oil fires, a phenomenon that mostly dissipated at redeployment.[11] More pertinent to the current conflict is the Al Mishraq sulfur fire that burned in Iraq for 3 weeks in June 2003. A retrospective cohort study indicated more self-reported respiratory symptoms on the Postdeployment Health Assessment, DD Form 2796 (administered within 3 months of the end of deployment), among exposed personnel but did not show an increase of chronic respiratory conditions in association with exposure to the smoke plume.[12]

Geological Dust

An analysis conducted by Englebrecht et al[13] on air samples collected from the CENTCOM AO implicated geological dust as one of the 3 main pollutants in the environment. The samples of interest showed generally expected composition, both chemical and mineralogical, when compared to samples from the Sahara and desert regions of the United States and China. However, average mass and chemical concentrations of the CENTCOM AO samples used in the analysis are as much as 10 times greater than those seen in samples from 10 rural and urban sites in the US near to military bases with similarly dry climates. Notably, the levels detected during this sampling program's efforts regularly exceeded the 24-hour standards set by the US Environmental Protection Agency.[14]

Exhaust and Industrial Byproducts

The operational setting in Iraq is largely desert, and industrial in nature. Existing infrastructure sources that contribute to air pollution include oil, cement, and fertilizer industries. The terrain of Afghanistan is mostly rugged mountains and desert, and environmental conditions have been described as degraded. Coupled with inherent environmental stressors of war, including factors like exhaust from military vehicles/heavy machinery and chemicals released during explosions, the extent of PM generated from these sources is of major concern regarding the respiratory health of our service members while deployed.[15-17]

Identification of Exposures Using Disease and Nonbattle Injury Data

Acute, high level exposures would likely affect few individuals, but are typically not reflected by sampling due to their unplanned nature. While individuals may seek

*The Institute of Medicine is the health component of the National Academy of Sciences. It is an independent, nonprofit organization that works outside of government to provide unbiased and authoritative advice to decision makers and the public. Information available at: http://www.iom.edu/About-IOM.aspx.

†The Institute of Medicine final report is discussed in detail in the article on page 43.

Table 1. Distribution of in-theater (OIF and OEF) toxic substance exposures (DNBI) requiring hospitalization from 2001 through mid 2011.

Toxic Substance Exposure	Environment When Exposed			Total
	Training	On-Duty	Unknown	
Poisoning by ingestion of toxic substances	1	414	14	429
Poisoning by inhalation of toxic substances	0	95	5	100
Adverse systemic or skin reaction by contact with a toxic substance	11	45	0	56
Total	12	554	19	585

Source: DoD Standard Inpatient Data Records database
OIF indicates Operation Iraqi Freedom
OEF indicates Operation Enduring Freedom
DNBI indicates disease and nonbattle injury

Table 2. Population demographics of Soldiers hospitalized in theater due to toxic inhalation exposures from 2001 to mid 2011 (N=100).

Category	No.
Age (years)	
17-19	6
20-29	62
30-39	24
40-49	6
50-59	2
Gender	
Male	93
Female	7
Rank	
E1-E3	25
E4-E6	64
E7-E9	1
W01-W05	3
O1-O3	6
O4-O1Ø	1
Component	
Active Army	67
Army Reserve	20
Army National Guard	13

Source: DoD Standard Inpatient Data Records database

care, these occurrences have not been systematically evaluated. Disease and nonbattle injury (DNBI) is the term for an illness and/or injury that is not directly related to enemy action or participation in direct combat. Reporting of DNBIs from deployed settings can be used to evaluate the frequency and nature of individual toxic inhalational exposures. They can include injuries and illnesses resulting from training or recreational activities or occupational and environmental exposures indirectly caused by military service. Throughout the history of US conflict, DNBIs have contributed significantly to decreased force strength and operational readiness of our fighting troops.[18-20] In order to highlight the impact of environmental exposures on the individual Soldier across the US Army, the focus of this article is DNBI related to toxic substance exposures, particularly those that are inhalational in nature, which resulted in hospitalizations or medical evacuations from the combat theater from 2001 through mid 2011. Since medical visits which did not result in hospitalization are not included, the data does not reflect exposures which may have been less severe.

DATA SOURCES

The DoD Standard Inpatient Data Records database stores information regarding inpatient medical encounters within the military health system. The electronic records for all hospitalizations occurring during deployment were accessed through the Patient Administration Systems and Biostatistics Activity. The US Transportation Command Regulating and Command and Control Evacuation System (TRAC^{2}ES) is a web-based data repository that stores information pertaining to all patient regulation and movement throughout DoD activities and locations.[21] Specifically, TRAC^{2}ES contains individual health-related data necessary to coordinate the transition of personnel requiring medical evacuation from the operational environment to a location where specialized medical care can be provided.[22] In-theater hospitalization data was reviewed to identify hospitalizations among active duty Soldiers due to toxic inhalation exposures between 2001 and mid 2011. External cause codes were reviewed and available records were further refined to identify additional information and need for medical evacuation.

RESULTS

Toxic Substance Exposure

Disease and nonbattle injuries related to toxic substance exposures are specified as one of the following 3 categories: poisoning by ingestion of toxic substances, poisoning by inhalation of toxic substances, or adverse systemic or skin reaction by contact with a toxic substance. The most commonly occurring subcategory is ingestion of toxic substances, followed by inhalation of toxic substances, then adverse systemic or skin reaction with a toxic substance. Table 1 shows the distribution for each toxic substance exposure category.

Hospitalizations

Demographics

The data for all US Army personnel with a history of OIF and OEF deployments between 2001 and mid 2011 were eligible for capture in the query for toxic inhalation-related hospitalizations and evacuations. A total of 100 such hospitalizations was identified. The distribution of population demographics for those hospitalizations is shown in Table 2.

Causes, Trends, and Theater Distribution

Specific causes (identified by ICD-9* codes in the primary diagnosis field) that attributed to toxic inhalational

*International Classification of Diseases, Ninth Revision

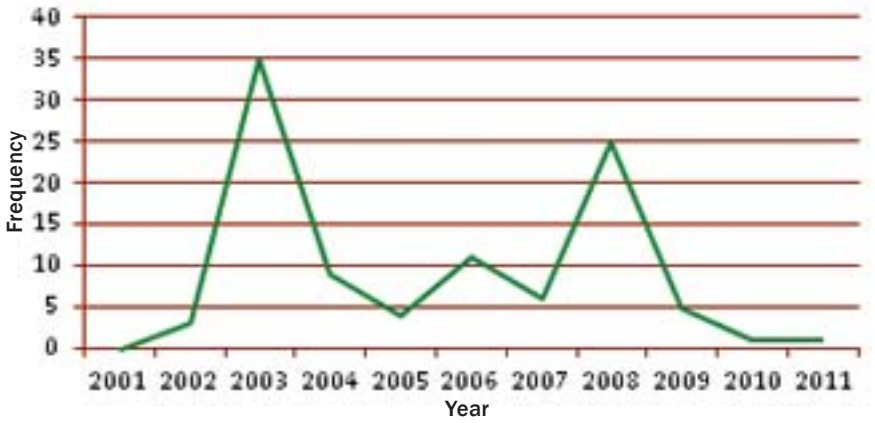

Figure 1. Yearly frequency distribution of toxic inhalation exposures requiring hospitalization (N=100) of Soldiers during Operations Iraqi Freedom and Enduring Freedom from 2001 through mid 2011. Source: DoD Standard Inpatient Data Records database.

exposures consisted mostly of toxic effects of gases, fumes, and vapors (not otherwise specified, n=22; not elsewhere classified, n=7), and toxic effects of chlorine gas (n=14). These diagnoses represent 43% of all toxic inhalational exposure hospitalizations (N=100). As shown in Figure 1, the frequency of toxic inhalational hospitalizations increased from 2001 through 2003, before decreasing and remaining fairly stable between 2004 and 2007, increased in 2008 before declining from 2009 through mid 2011. Operation-specific counts indicate the majority (n=91) of toxic inhalational exposure hospitalizations occurred in OIF, with 9 hospitalizations recorded from OEF.

Evacuations

Data gathered from TRAC²ES indicated 26 air evacuations from the combat theaters of OIF and OEF between 2001 and mid 2011 with toxic inhalational exposure listed as the cause of injury. The population demographics for those evacuations is presented in Table 3.

As shown in Figure 2, the frequency of evacuations for toxic inhalation increased from 2001 through 2004, decreased in 2005 before increasing and levelling between 2006 and 2008, then declined in 2009 and remained fairly stable through mid 2011. Theater-specific counts indicate the majority of toxic inhalational exposure evacuations were made from OIF (n=20), with 6 evacuations recorded from OEF.

CONCLUSION

Disease and nonbattle injuries have contributed significantly to overall morbidity and mortality associated with battle throughout the history of US conflict, and the current conflicts are no exception.[18,20] Based on the available data, poisoning by exposure to toxic substances, including toxic inhalations, is relatively infrequent. Some cases are serious enough to warrant in-theater hospitalization, a subset of which results in out-of-theater medical evacuation.

While deployed in support of OIF and OEF, our military personnel may experience toxic substance exposures that can have respiratory health consequences. Unfortunately, most of these exposures in the deployment environment cannot be avoided, and the demands of combat operations and wartime stressors may hinder adequate preventive measures. While both the acute and chronic health effects of such exposures are not yet fully understood, studying such a relationship is difficult due to a lack of individual exposure data. Hospitalization and medical evacuation data related to toxic inhalational exposures were examined in an effort to better understand the extent of true exposure during deployment at the individual level in an objective fashion. Available data indicate that the number of toxic inhalational exposures significant enough to require hospitalization from 2001 through mid 2011 is small relative to the number of troops who were deployed and potentially exposed during that time period. A smaller subset of the hospitalized service members required medical evacuation due to severe inhalational exposures. As the data used in this study were limited to those toxic exposures significant enough to require hospitalization or medical evacuation, it represents some fraction of a still unquantified denominator that includes exposures that may have resulted in symptoms or medical evaluation, but not hospitalization. Additionally, toxic inhalation exposures may affect individuals with conditions which make them more

Table 3. Population demographics of Soldiers evacuated from theater due to toxic inhalation exposures from 2001 to mid 2011 (N=26).

Category	No.	%
Age (years)		
17-19	1	3.8
20-29	14	53.8
30-39	9	34.6
40-49	2	7.7
Gender		
Male	22	84.6
Female	4	15.4
Rank		
E1-E3	15	57.7
E4-E6	9	34.6
01-03	2	7.7
Component		
Active Army	16	61.5
Not annotated	10	38.5

Source: US Transportation Command Regulating and Command and Control Evacuation System

susceptible to exacerbations, such as asthma.[23] These hospitalizations would most likely be coded with the primary condition (asthma) and not the precipitating exposure. Therefore, while available data indicate that acute inhalational injury of significance is a rare event, this likely represents the "tip of the iceberg" of inhalational exposures while deployed.

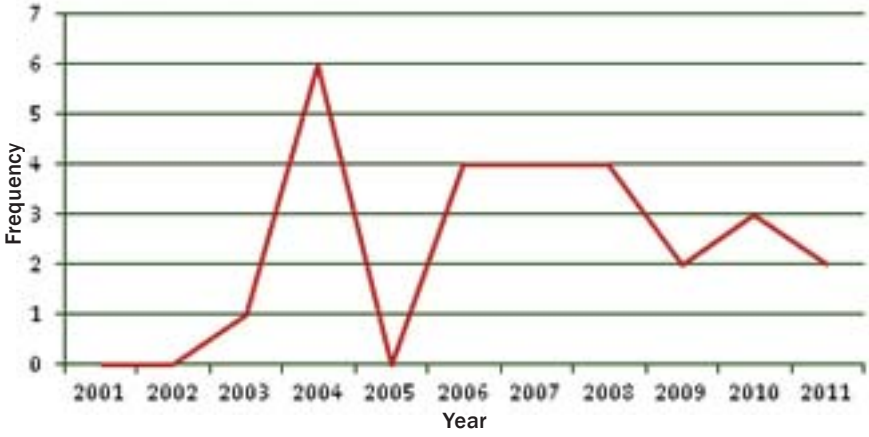

Figure 2. Yearly frequency distribution of toxic inhalation exposures requiring medical evacuation (n=26) of Soldiers from the combat theaters of Operations Iraqi Freedom and Enduring Freedom from 2001 through mid 2011. Source: US Transportation Command Regulating and Command and Control Evacuation System

The concern that the DoD has not truly identified the full impact of toxic inhalational exposures during military operations is not new, however. In fact, some personnel have received treatment due to environmental exposures during deployment, yet systems previously available have not captured this information adequately. As a result, some very specific solutions have been recently developed to improve the quality of the data collected when exposures result in medically notable encounters. These efforts include the establishment of the Defense Occupational and Environmental Health Readiness System-Incident Reporting module,* an official DoD archive system that includes fields for collection of incident data, personnel rosters, and medical/duty status; the institution of specific ICD-9 causal codes for consistent reporting and surveillance; development of base camp periodic occupational and environmental monitoring summaries and incident/hazard specific factsheets. Additionally, the US Army Public Health Command's Environmental Medicine Program now offers an Environmental Medicine Clinical Consult Service, the only official environmental medicine level V support† offered through the Army Medical Command which provides documents and presents recommendations regarding diagnostics and/or medical documentation to address individual concerns associated with environmental exposures (see inset below). These tools facilitate more comprehensive evaluation, documentation, and reporting of toxic inhalational events and other environmental exposure incidents, and improve the DoD's ability to address post deployment health concerns related to such exposures.

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*https://doehrs-ih.csd.disa.mil/Doehrs/

†Level V is support provided by the US Army Public Health Command and the Navy and Marine Corps Public Health Center. Responsibilities include, but are not limited to, supporting deployed level I – IV preventive medicine personnel; performing definitive testing of air, water, and soil samples; and performing vector pathogen testing.[24]

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The Impact of Attachment Style on Posttraumatic Stress Disorder Symptoms in Postdeployed Military Members

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ABSTRACT

This study examined the effects of attachment style on self-reported posttraumatic stress disorder (PTSD) symptoms in a population of service members (N=561). Active duty, postdeployment service members completed anonymous questionnaires including 2 measures of adult attachment and the PTSD checklist–military as a measure of PTSD symptoms. Results confirmed the central hypothesis that attachment style was related to reported PTSD symptoms. Secure attachment style was associated with less reported PTSD symptoms and therefore may be involved in mechanisms associated with protection from developing PTSD after experiencing wartime trauma. Results were consistent when tested across continuous and dichotomous assessments that captured diagnostic criteria. This study demonstrates a significant relationship between attachment style and PTSD symptoms within a military population, potentially providing the basis for future research in this area.

Throughout history there have been psychological effects on Soldiers and their Families during combat deployment.[1] Posttraumatic stress disorder (PTSD) is an anxiety disorder that may occur following an emotionally terrifying, life-threatening event or events that create psychological trauma. Events associated with onset of PTSD include, but are not limited to:

> military combat, violent personal assault (ie, sexual assault, robbery, mugging), being kidnapped or taken hostage, terrorist attack, torture, incarceration as a prisoner of war or in a concentration camp, natural or manmade disasters, and automobile accidents.[2]

It is estimated that 5% to 24% incidence of PTSD for the over 2 million American troops deployed to Iraq and Afghanistan occurred from September 2001 until October 2009.[3] Primary characteristics of PTSD are debilitating fear and helplessness.[4] As such, severe PTSD symptoms can be detrimental to the overall life and functioning of the individual, with consequences at the biological, psychological, and social levels. The social implications of PTSD directly relate to attachment theory and the disruption of ways we relate with others in our social support system.[5] Attachment theory provides a framework for understanding and addressing the central problems of PTSD that affect psychosocial functioning: emotion or affect regulation, interpersonal skills, and social support behaviors.[6,7]

ATTACHMENT THEORY AND ATTACHMENT PATTERNS/STYLES

Adult attachment is an extension of the early attachment relationship between the infant and caregiver.[8] This relationship sets the foundation for all future attachment relationships and the "internal working model" of self and of others. The theory concentrates on secure attachments between infants and their caregivers as related to the development of social and emotional stability. Moreover, the ideal of secure attachment "assumes that successful navigation through the universal stages of attachment normatively provides children with a secure emotional attachment base, a base from which children competently lead the rest of their relational lives."[9]

Child attachment theory was developed in the 1970s by Mary Ainsworth,[10] who established 3 different attachment styles in children: type B or secure, type A or avoidant, and type C or ambivalent/resistant. A fourth category identified by Main and Solomon[11] was labeled as disoriented or disorganized attachment, or type A/C.[8] The field was further developed by Bowlby[8] who asserted the first attachment relationship between the infant and caregiver (usually the mother) sets the stage for all future attachment relationships. In their studies of romantic love, Hazan and Shaver[13] developed a 3-category theory of adult attachment based on Ainsworth's original 3 infant-parent styles. Their styles were labeled secure, avoidant, and ambivalent. As with the child literature, a fourth

adult attachment category was added by Bartholomew.[13] Bartholomew's styles are secure, preoccupied, fearful, and dismissing. Conceptually, the secure and preoccupied styles are similar to Hazan and Shaver's[12] secure and ambivalent styles, whereas fearful and dismissing describes 2 different types of avoidant individuals. More recently, the adult attachment literature has expanded to look at adult attachment more succinctly as a composite of relationship anxiety and relationship avoidance.

Attachment style is based on how you feel about yourself and about others. In Bartholomew's styles, secure describes low relationship anxiety and low avoidance, preoccupied indicates high anxiety and low avoidance, fearful depicts high anxiety and high avoidance, and dismissing characterizes low anxiety and high avoidance. Additionally, insecure adults may have anxious-resistant attachment, which means they worry that their partner may not love them completely, and they are emotionally reactive when their attachment needs go unmet. Conversely, avoidant partners appear not to care too much about close relationships; they are not dependent on others and others cannot be dependent on them.[12] The attachment research literature shows that individuals with secure attachment "score higher on personality variables indicative of self-confidence, psychological well-being, and functioning in the social world."[15] Securely attached individuals are also described as "adaptive, capable, trusting and understanding," as well as "able to appraise stressful situations, cope more positively with them, and adjust more flexibly to these experiences."[14]

POSTTRAUMATIC STRESS DISORDER AND SOCIAL BONDS

Interpersonal factors play a large role in the diagnosis, development, maintenance, and recovery from PTSD. From a diagnostic perspective, symptoms of social impairment include various degrees of withdrawal from relationships and social roles. In terms of development, PTSD diagnoses often result from interpersonal trauma, such as rape and abuse, as compared with natural disasters, or even the trauma of combat itself. As such, it appears that PTSD involves a dissembling of the internal structures of trust and attachment that allow us to connect with important others and to function normally in social settings as a result of this breach in social bonds via trauma. Regardless of the kind of traumatic experience, people with PTSD suffer extreme social difficulty due to the impairment to the ability to distinguish between dangerous and normal stimuli.[16] Trauma studies show that the biophysical, psychological, and social functioning of individuals with PTSD is comprised at neurophysiological levels in such a way that limbic systems for self regulating or self-calming are disrupted; rational thinking and action are debilitated; and interpersonal relationships as well as social bonds are often broken. It is important to note that social support processes are at play within these sequelae of PTSD and the severity of symptoms.[17] People with PTSD have difficulty drawing on social support when they need it most.[18] And in turn, resources of social support tend to diminish as people with PTSD are unable to reach out for help.[19] Several studies show that social support is an important factor in adjustment and functioning for Veterans with PTSD.[20,21] While severity and prognosis are varied, the impact on military performance, family, and quality of life has precipitated significant clinical and research interest.

Closely related to social support, particularly through the lens of attachment theory, is the experience of intimate partner relationships. Importantly, intimate partner relationships are also known to be an important factor in overall functioning for Veterans and Soldiers, if not for all families.[22] This area of research provides a particularly informative application of attachment theory in light of attachment styles with adult romantic partners, which is considered by current attachment theory to be an extension of the individual attachment style established with the primary caregiver.[23] Recent research shows that this theory is supported in its application to dyadic, or couple's processes in PTSD outcomes.[24-27] This growing body of research shows that PTSD is associated with insecure attachment styles.[27,28] Additionally, recent studies have shown that marital functioning and couple adjustment is an important aspect for Veterans and Soldiers with PTSD.[24,29] Two recent studies show that marital satisfaction plays an important role in lower symptom severity of Veterans with PTSD.[30,31] This theoretical perspective is beginning to provide insight into the interpersonal factors at work in PTSD outcomes, making this an opportune time to further explore relationships between mechanisms of attachment and PTSD in recent Veterans.[24-26] Posttraumatic stress disorder has recently been increasingly associated with attachment theory due to the interpersonal nature of the disorder.[32]

THE CURRENT STUDY

Data from a cross-sectional study were analyzed to further explore the relationship between attachment styles and PTSD. Of note, this study examined the relationship between PTSD symptoms and 2 different but theoretically and empirically related assessments of human attachment. Regarding the first assessment, our first hypothesis was that PTSD symptoms would be differentially related to each of the categorical attachment measure styles. We expected the higher PTSD scores to be associated with the fearful group and the lower PTSD scores to be associated with the secure group.

It is also hypothesized that relationship anxiety and relationship avoidance would predict reported PTSD scores with low relationship anxiety and low relationship avoidance being related to lower PTSD scores with the opposite being related to higher PTSD scores.

METHODS

Procedure

Data were collected as part of a quantitative, cross-sectional study looking at attachment, temperament, and resilience as protective mechanisms for posttraumatic stress. Data were collected on anonymous questionnaires distributed on Fort Sam Houston and Lackland Air Force Base in San Antonio, Texas, from summer 2010 to summer 2011. In order to participate in this study, the participants must have been deployed for at least 30 days or more, aged 18 years or older, and on active duty. The study was reviewed and received an exempt determination from the Brooke Army Medical Center's Institutional Review Board. For this study, the independent variable was adult attachment (both the categorical attachment measure (RQ) and the continuous measure of adult attachment (see description in Attachment section below), and the dependent variable was PTSD symptoms.

Participants

Among the 561 respondents, 403 were male, 157 female, and one no response; 8% aged 25 years and younger, 23% in the 26 to 30 year age range, 48.5% aged 31 to 40 years, 21% 41 years of age and over; 69% married or living with a partner; 62% Army and 37% Air Force; 54% SGT, SSG, or SFC; 23% LTs to CPTs*; 22% with master's degree or higher, 30% with bachelor's degree, and 44% had some college. The ethnicity of the sample was 12.3% Hispanic and 86.6% non-Hispanic; the racial profile was 65.6% white, 19.6% African American; 5.9% Asian/Pacific Islander, and 8% other. All participants had deployed at least once. Each participant reported personal total career deployment time. resulting in an average of 1.9 years (1 year, 10.8 months), ranging from one month to 14 years.

MEASURES

Attachment

Adult attachment was measured 2 ways: one with the Bartholomew and Horowitz Relationship Questionnaire,[33] a 4-item categorical adult attachment variable; the other with the Fraley et al[34] Experiences in Close Relationships [scales]-Revised, (ECR-R) which creates continuous anxiety and avoidance attachment variables. The conceptual relationship between the categorical measure of adult attachment and the continuous measure is that secure adults are low in relationship anxiety and avoidance; fearful adults are high in relationship avoidance and relationship anxiety; the preoccupied adults are low in relationship avoidance and high in relationship anxiety; whereas dismissing are higher in relationship avoidance and lower in anxiety. Shaver and Fraley[35] further developed the relationship between these 2 self-report measures of adult attachment.

Experiences in Close Relationships-Revised[34] is a measure of adult attachment. This is a 36-item self-report instrument designed to measure attachment-related anxiety and avoidance. Participants are asked to think about their close relationships, without focusing on a specific partner, and rate the extent to which each item accurately describes their feelings in close relationships, using a 7-point scale ranging from "not at all" (1) to "very much" (7). Eighteen items tap attachment anxiety and 18 items tap attachment avoidance. Internal consistency reliability tends to be 0.90 or higher for the 2 ECR-R scales.

The Relationship Questionnaire[33] is a self-report adult attachment measure. The measure includes a series of 4 statements that represent secure, preoccupied, fearful, and dismissing adult attachment styles. Participants are instructed to place a checkmark next to the letter corresponding to the style that best describes themselves. Next they are asked to rate each of the presented relationship styles to indicate how well or poorly each description corresponds to their general relationship style as measured by a Likert-type scale, from "disagree strongly" to "agree strongly." Test-retest reliabilities of the RQ subscales ranged from 0.49 to 0.71 as were reported by Scharfe and Bartholomew.[36] Schmitt and colleagues[9] validated the attachment questionnaire in 62 cultures suggesting that people worldwide fall into one of the 4 attachment patterns, and there are cultural differences that suggest societal norms influence one's resulting attachment pattern.

Posttraumatic Stress Disorder Symptoms

The PTSD checklist–military,[37] commonly known as the PCL-M, is a 17-item self-report inventory that assesses the severity of each DSM-IV†-defined PTSD symptom. Each item corresponds to the DSM-IV diagnostic criteria for PTSD and is scored on a 1 (not at all) to 5 (extremely) scale. Previous research on the PCL-M indicated mean scores of 64.2 (SD=9.1) for PTSD subjects and 29.4 (SD=11.5) for non-PTSD subjects.[37] The

*SGT indicates sergeant; SSG indicates staff sergeant; SFC indicates sergeant first class; LT indicates 1st or 2nd lieutenant, CPT indicates captain.

†*Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition[38]

PCL is widely used in the Department of Defense and the Department of Veterans Affairs and has excellent reliability and validity.[37]

DATA ANALYSIS

The data analysis was conducted using SPSS version 18 (SPSS, Inc., Chicago, IL). An analysis of variance (ANOVA) was used to test the first hypothesis, which examined the relationship between the RQ and the PTSD scores. For further analysis, the PTSD score was dichotomized creating a categorical variable of low and high PTSD. A logistic regression was used to test the second hypothesis, which examines the relationship between the ECR-R and the PTSD scores.

To test the validity of using our current measures in this population, we examined the relationship between the RQ and the ECR-R to determine the conceptual relationship between these instruments. Using this sample, our results were consistent with the literature. Those who selected the secure attachment style also rated themselves as lower avoidance and lower anxiety compared to fearful, preoccupied, and dismissing; fearful rated themselves as higher anxiety and avoidance than secure, preoccupied, dismissing, etc. In a separately published article,[39] we present a more detailed discussion of the relationship between the RQ and ECR-R.

RESULTS

Descriptive Statistics

The RQ is made up of 4 possible attachment styles: secure, fearful, preoccupied, and dismissing. In our sample, 39.3% selected secure, 24% fearful, 7.2% preoccupied, and 29.5% dismissing as their attachment style. The ECR-R creates 2 measures of attachment, relationship anxiety and relationship avoidance. The mean scores on each subscale were 2.79 for anxiety and 2.79 for avoidance with standard deviations of 1.21 and 1.15 respectively. The PTSD Score on the PCL-M ranged from 17 to 76 with a mean of 30.23 (SD=14.40). Higher scores on the PCL-M indicate more reported PTSD symptoms. 13% of our sample scored 50 or over on the PCL-M whereas 33% of our sample scored 32 or higher.

Attachment Style and Posttraumatic Stress Disorder

An ANOVA was conducted using the self-selected attachment style (secure, fearful, preoccupied, or dismissing) as the independent variable and the PTSD score as the dependent variable. Least squared difference was used for the follow-on contrasts. This resulted in a significant ANOVA, $F_{3,501}=18.05$; $P<.001$, and in significant differences between all attachment styles except for the preoccupied and dismissing styles (Figure 1). The means (M) and standard deviations for the PTSD scores on the RQ measures resulted for secure (M=25.57, SD=10.86), fearful (M=37.14, SD=16.28), preoccupied (M=31.83, SD=14.53) and dismissing (M=30.24, SD=14.55).

In our second analysis, we examined diagnostic implications for PTSD. In order to dichotomize PTSD cases, we used a cutoff of 32 on the PCL score which is consistent with a screening threshold for this self-report measure. A score of greater than or equal to 32 is considered to have a higher sensitivity than the 50 or higher cutoff traditionally seen in research.[40] Although there is some debate, researchers recommend using a cutoff score between 30 and 34 when using the PCL.[41]

Chi-square analysis was conducted using the dichotomous PTSD variable of low versus high PTSD severity score. Low PTSD severity scores included scores from 17 to 31, whereas high PTSD severity score category included scores from 32 to 76. Twice as many individuals were classified by having a low PTSD severity score (66.7%) as compared with those classified as having a high PTSD severity score (33.3%). The Chi-square analysis resulted in significant differences ($\chi^2=40.343$, $P=.000$, N=502).

Figure 2 demonstrates that secure attachment produces lower frequencies in the high PTSD severity category and the fearful style produces the greatest frequencies, followed by preoccupied and then dismissing. Conversely, the secure style has the greatest representation in the low PTSD severity category.

We predicted that individuals reporting lower relationship anxiety and avoidance would predict lower levels of PTSD scores. A *t* test relationship anxiety and

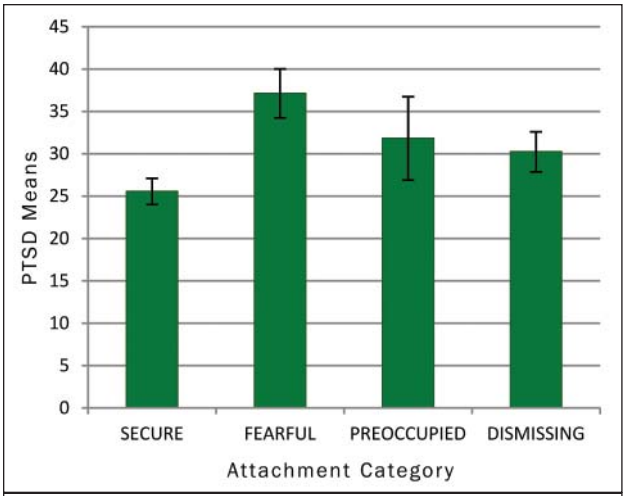

Figure 1. The mean posttraumatic stress disorder scores plotted by attachment style.

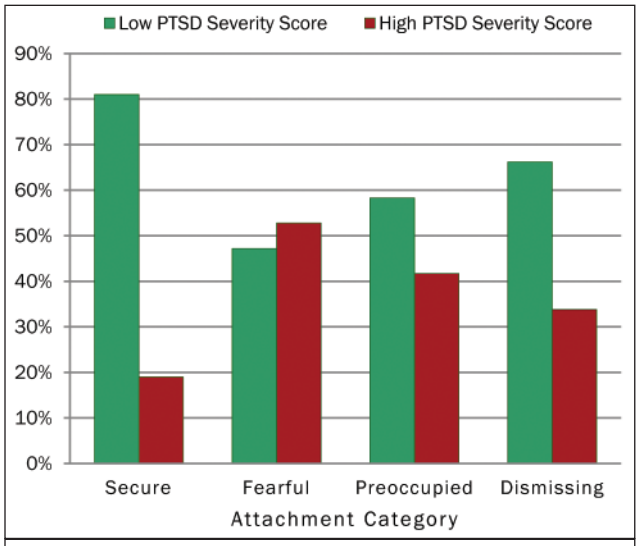

Figure 2. Attachment style plotted by dichotomous posttraumatic stress disorder score.

relationship avoidance based on whether they were in the low or high PTSD severity category. This resulted in a t_{539}=-7.63, P<.001 for relationship anxiety and a t_{538}=-8.79, P<.001 for relationship avoidance. The low PTSD severity score group had a mean of 2.53, SD of 1.10 on relationship anxiety and a mean of 2.50, SD of 1.03 for relationship avoidance, whereas the high PTSD severity group had a mean of 3.33, SD of 1.26 for relationship anxiety and a mean of 3.37, SD of 1.17 for relationship avoidance. Our results, shown in Figure 3, indicate that individuals who self report low levels of PTSD symptoms also report significantly lower levels of relationship anxiety and relationship avoidance than those who self report high levels of PTSD symptoms.

COMMENT

Adult Attachment and Service Members

Descriptive statistics showed that approximately 40% of our population of service members is self-classified as securely attached individuals. The rest are self-classified as one of the insecure attachment styles (ie, fearful, proccupied, dismissing). Research outcomes supported our hypotheses that securely attached individuals report far fewer incidences of PTSD outcomes on both categorical and continuous measures of attachment. More severe symptoms were associated with less functional attachment styles, and less severe with more functional styles. These findings were strengthened by the consistency across the two different types of attachment measurement, one a self-reported style and the second measure a detailed description of relationship functioning. Thus, these outcomes provide insight into both the intrapersonal and interpersonal aspects of the attachment system as it pertains to this population.

Our prevalence rates of PTSD when defined as scoring 50 or higher on the PCL-M (13%) were consistent with the prevalence rates in the literature (13%) for service members returning from the wars in Iraq and Afghanistan.[42] Additionally, our results were consistent with the known relationship between attachment style and PTSD outcomes in other high-risk populations. These findings have important implications to our military population at the individual and organizational levels. Understanding attachment patterns and styles among service members can possibly be both a protective factor and a diagnostic factor in mitigating the risk of PTSD and providing treatment to service members and their families. Additionally, attachment measures may help guide recruitment, placement, and organizational decisions for the military.

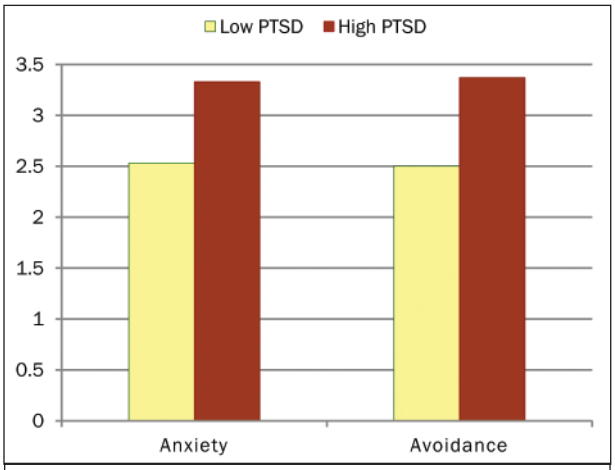

Figure 3. Relationship anxiety and avoidance plotted by dichotomous posttraumatic stress disorder score.

Adult attachment style may protect service members from developing PTSD after experiencing combat and combat-related experiences. Attachment theory asserts that "any relationship in which proximity to the other affects security is an attachment relationship"[43] and therefore most all professional relationships in the military impact the individual attachment system. Moreover, an attachment relationship does not have to be a romantic relationship and may be any relationship such as peer to peer, subordinate to supervisor, leader to follower, or same or mixed gender relationships. By the time a person enters the military their propensity for certain attachment styles has been established and may play a role in how much trust is placed in new relationships (ie, peer to peer, leader to follower, etc). As early as basic training young trainees are assigned a battle-buddy* and encouraged to always have their battle buddy with

*Generally defined as the person to whom a Soldier can turn in time of need, stress, and emotional highs and lows who will not turn the Soldier away, no matter what. This person knows what the Soldier is experiencing because of experience with similar situations or conditions, either current, previous, or both.

them. When assigned to a military unit, especially in a stressful combat environment, relationships with others within the unit are vital to enabling a cohesive effort towards a collective goal. Many view the others within their unit as their "military family," and are encouraged to always have a battle buddy or a "wingman" and support each other, establishing positive relationships throughout their career in the military, and some even follow beyond retirement. Conversely, there are unstable relationships within units, sometimes causing detrimental effects, especially when individual members isolate themselves, inhibiting communication, and consequently harming unit cohesion and effectiveness, not only for themselves, but for their entire unit.[43]

Military personnel with secure attachments, especially with their respective military family, appear to experience less stress because they use social coping mechanisms. They are more apt to engage with their families and peers, and go to mental/behavioral health practitioners or the chaplain for assistance, all of which mitigates the risks to developing symptoms of PTSD. Flexibility allows these securely attached individuals to adapt well to their environment. Beyond preventing PTSD, secure attachment may also contribute to the reconstruction of comforting, health sustaining beliefs shattered by trauma, an example of what Tedschi and Calhoun[44] call posttraumatic growth. Current efforts by the military have focused on group debriefings, psychotherapy, and psychopharmocological interventions. However, additional efforts could be focused on making a more successful match between treatment approaches so that those who are not securely attached can receive supportive interventions that may prevent the symptoms of PTSD. Based on these various attachment styles, providers would be able to plan programs and provide interventions and treatments for service members in the predeployment, deployment, or postdeployment phases.

FUTURE DIRECTIONS

The relationship between attachment style and PTSD outcomes in service members clearly merits further inquiry. Future studies will need to explore the subcategories of the PTSD diagnosis with respect to attachment styles in order to show more specifically how the attachment system affects the disorder. More detailed information on these relationships can guide the development of programs and interventions, and inform the application of attachment related treatment to the clinical context. Additionally, longitudinal studies examining the relationship between these variables pre- and posttreatment and pre- and postdeployment will advance the determination of causal factors, the potential for change, and the efficacy of prevention measures. For example, is attachment style changed by trauma or is it more of a risk factor? If something can be done in the military to help promote secure attachment in the interest of strengthening our forces, what could that be and how can this be undertaken within a military setting?

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US Department of Defense photo

A Review of Mechanics and Injury Trends Among Various Running Styles

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ABSTRACT

Context: Running related overuse injuries are a significant problem with half of all runners sustaining an injury annually. Many medical providers and coaches question how to advise their running clients to prevent injuries. Alternative running styles with a more anterior footstrike such as barefoot running, POSE running, and Chi running are becoming more popular. Little information, however, has been published comparing the mechanics and injury trends of different running styles.

Objective: The original purpose of this paper was to examine evidence concerning the biomechanics and injury trends of different running styles. Little to no injury data separated by running style existed. Therefore, we discuss the biomechanics of different running styles and present biomechanical findings associated with different running injuries.

Data Sources: English language articles published in peer reviewed journals were identified by searching PubMed, CINAHL, and SPORTDiscus databases. Nearly all of the studies identified by the search were observational studies.

Results: A more anterior initial foot contact present in barefoot or other alternative running styles may decrease or eliminate the initial vertical ground reaction peak or "impact transient," possibly reducing knee joint loads and injuries. A more anterior foot strike, however, may increase mechanical work at the ankle and tensile stress within the plantarflexors. Wearing minimal footwear may also increase contact pressure imposed on the metatarsals.

Conclusion: More research is needed to determine which individuals with certain morphological or mechanical gait characteristics may benefit from alternative running styles that incorporate a more anterior initial foot contact with or without shoes.

The popularity of running is at an all-time high with nearly 500,000 people in the United States completing a marathon in 2009.[1] Annual running injury incidence has recently been reported between 19% and 79%.[2] This large number of injuries has medical providers and coaches struggling to determine how best to advise their running clients to prevent injuries. Alternative running styles such as barefoot running, POSE running, and Chi running have enjoyed an increase in popularity recently. Proponents of these alternative running styles with a more anterior landing pattern claim that employing these techniques will reduce injuries. Little information, however, has been published comparing the mechanics and injury trends associated with different running styles.

OVERVIEW OF DIFFERENT RUNNING STYLES

Traditional Shod Running

A recent kinematic analysis of elite runners wearing shoes who participated in a half marathon indicated that 75% of the runners were heel strikers, 24% were midfoot strikers, and 1% were forefoot strikers.[3] When runners use a rearfoot strike pattern, the knee is relatively extended and the ankle is in relative dorsiflexion upon initial contact. As the ankle moves into plantarflexion, the knee flexes and the knee extensors act eccentrically to dampen the ground reaction forces. Traditionally shod rearfoot strikers often take long strides, characterized by a vertical displacement of the center of mass and an impact peak present at approximately 10% to 12% of the stance phase on the vertical ground reaction force curve (Figure 1).[4] Runners using a rearfoot strike pattern in bare feet or minimalist footwear have demonstrated greater initial vertical loading rates than shod heel strikers.[4,5] Runners using a rearfoot strike may require greater angular work at the knee[6] resulting in higher patellofemoral and tibiofemoral compressive forces[7,8] and possibly greater risk of knee injury than other running styles with more anterior footstrike patterns. Advocates of barefoot and alternative running styles report that initial heel contact running is a relatively new phenomenon associated with the development of the modern running shoes with thicker cushioned heels in the last 30 to 40 years. Prior to this, many believe the proportion of midfoot and forefoot strikers was much greater.

Alternative Running Styles

Barefoot running and other alternative running styles have gained recent popularity, leaving many health care providers with questions regarding the safety and appropriateness of these techniques for various running populations. In several publications, barefoot runners exhibited a more anterior midfoot or forefoot striking pattern, thereby avoiding heel strike.[4,9-11] A growing number of barefoot running advocates, teachers, and websites have provided barefoot running instruction since publication of McDougall's 2009 book.[12] Generally with habituated barefoot runners, stride length is shortened, stride frequency is increased, and the vertical displacement of the center of mass is reduced.[9,13,14]

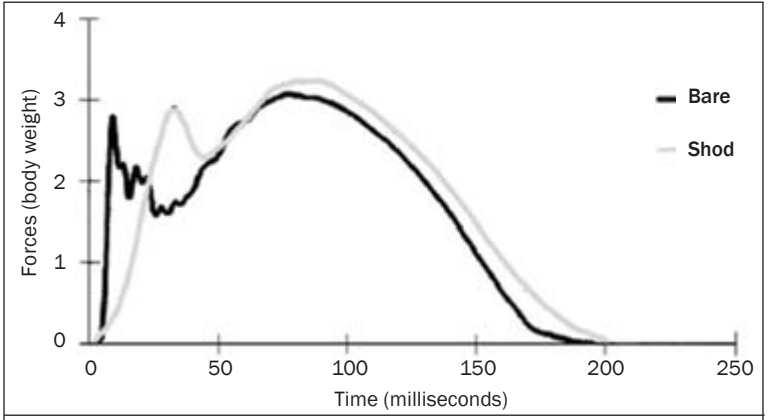

Figure 1. Vertical ground reaction curves of 1 representative person demonstrating a rearfoot strike pattern in bare feet and shod. Reprinted from De Wit et al[4] with permission.

One alternative running style that has gained popularity recently is the POSE method designed by Dr Nicolas Romanov.[15] This running strategy involves a midfoot to forefoot strike pattern that minimizes contact time with the support surface and focuses on picking up the feet and not pushing off the ground as vigorously.[15,16] Romanov claims that gravity causes the muscle system to absorb body weight on landing during POSE running, which then produces elastic strain energy. Romanov further describes that as the center of mass passes over the support limb, a gravitational torque occurs as extensor muscle activity ceases. The runner falls forward while the ground reaction forces decrease and vertical work against gravity is reduced. Romanov suggests that the foot is unweighted during terminal stance, as it is rapidly pulled from the ground by hamstring muscle activity to reduce lower-limb inertia and to catch up with the body. The focus on falling via a gravitational torque and pulling the foot from the ground effectively differentiates POSE running from more traditional running forms.

Another alternative running style that has recently gained popularity is Chi running. The founder of Chi running, Danny Dreyer, credits the origins of this running form to the discipline of Tai Chi.[17,18] This method of running is described as the alignment of body, mind, and forward movement. Runners are instructed to avoid heel strike and to land with a midfoot strike pattern. The body leans forward slightly, and the strides are shorter with a focus on relaxed legs. Dreyer recommends that runners discard more traditional heavily padded running shoes and use a more minimalist running shoe that involves thin sole material and limited supportive features.[17,19] In summary, barefoot, POSE, and Chi runners attempt to land with a midfoot or forefoot strike, take shorter strides with a greater frequency, and may demonstrate a reduced initial vertical ground reaction impact compared with traditional heel-toe shod runners. The original purposes of this review of literature were to examine additional evidence concerning the kinematics, kinetics, and injury trends associated with different running styles. Little to no injury data separated by running styles were found. Therefore, we discuss the biomechanics of different running styles and present biomechanical findings associated with different running injuries.

DATA SOURCES

English language articles published in peer reviewed journals were identified by searching PubMed, CINAHL, and SPORTDiscus databases. Key words used in this search included running, barefoot, POSE, Chi, kinematics, kinetics, injury, and running styles in various combinations. The authors included original research, meta-analyses, and review articles in the search. Only one randomized control trial was identified.[20] Nearly all of the studies included were observational studies. The search for manuscripts detailing aspects of Chi running in scientific peer reviewed literature yielded no results. The authors then resorted to using popular literature and website descriptions of the Chi running style.

RESULTS

Running Mechanics

Traditional Shod Running

For heel strikers (approximately 80% of shod runners),[3,9] the initial (impact) peak vertical ground reaction force at heel strike occurs during the first 10% of stance[21-23] or within approximately 25 milliseconds.[24] This force is passive in nature and the anterior-posterior component of this impact is generally considered a braking

force with the heel strike anterior to the runner's center of mass. The second peak for the vertical ground reaction force occurs between 40% and 50% of the stance phase.[25] This force is more active as the runner pushes off the ground and the anterior-posterior component is more propulsive in nature with the runner's center of mass superior/anterior to the foot contact. Typical running peak vertical ground reaction forces for runners are between 1.5 and 3.5 times body weight.[25] Vertical ground reaction forces increase linearly with increasing running velocity[26] and increasing stride length,[27-29] and decrease with a faster stride rate or cadence.[30] Runners with a history of injuries may demonstrate greater initial peak vertical ground reaction forces than healthy matched runners,[22,23,31] however, this point has been refuted.[32]

Cushioned running shoes are commonly prescribed for runners with high arches and motion control shoes are often recommended for low arched runners who require pronation control. Cushioned running shoes may attenuate the ground reaction force better for high arched runners[33] and motion control shoes may control instantaneous loading rates better for low arched runners.[34] Increased resultant joint torques at the hip and knee have been observed in shod runners compared with barefoot runners.[7] Aside from the effects of footwear modifications, some runners may benefit from an altering their running style and learning to run with a reduced impact load or ground reaction force.[21] However, this has not been widely studied to date.

Alternative Running Styles

Most habitual barefoot runners choose to land with a midfoot or forefoot initial foot contact to avoid greater initial loading rates observed with heel striking in barefeet[4] (Figure 1). While most runners attempting to run in bare feet or minimalist shoes will convert to a more anterior footstrike, McCarthy et al reported recently on a sample in which 50% of runners continued to demonstrate a rearfoot strike pattern 2 weeks after changing to the Vibram 5-finger shoe.[5]

A toe-heel-toe or midfoot contact pattern used by barefoot runners and other minimalist shoe runners who use this landing strategy may decrease the vertical loading rates and initial passive peak vertical ground reaction force by 15% to 33% during the first 25 milliseconds of foot contact compared to traditional heel-toe strike patterns.[9,35] This reduction in initial peak vertical ground reaction force is accomplished by prolonging the time needed to decelerate the runner's vertical velocity after initial foot contact. By prolonging this period of time with a greater ankle range of motion,[10] the vertical ground reaction force is reduced as reflected by the impulse-momentum equation $F = m \times \Delta v / \Delta t$, where F = vertical ground reaction force, m = mass of runner, Δv = the change in vertical velocity from initial foot contact to the velocity of zero when downward motion stops, Δt = the time required to change the downward velocity to zero.

The period of time required to change a runner's downward velocity to zero (Δt) will likely be longer with a toe-heel-toe initial contact pattern than with a heel strike pattern. The initial vertical ground reaction force (F) will therefore be reduced. Another mechanism to decrease vertical ground reaction forces given a fixed mass would be to reduce the amount of change in velocity. This can be accomplished by reducing the vertical height from which the body's center of mass falls to the ground.[36] Essentially, limiting the vertical displacement of the center of mass prior to foot contact will reduce Δv. This is achieved by adopting running styles in which the runner glides forward more and bounces up and down less.

Little research has been conducted concerning injury trends that are associated with barefoot or other alternative running styles. Particularly of concern to some medical providers are metatarsalgia and other injuries related to foot contact patterns, particularly in bare feet.[37,38] Injuries caused by excessive contact pressures that are perpendicular to the foot-ground interface are governed by the equation

$$\text{contact pressure} = \text{contact force} / \text{contact area}$$

Wearing minimal footwear that has relatively thin sole material and no supportive features built into the shoe's construction may simulate conditions of barefoot running.[39] Running in bare feet or using minimal footwear may increase peak contact pressure, increase maximum ground reaction force, and reduce contact area of the foot, thereby increasing peak pressures imposed on the forefoot.[40,41] For a given ground reaction force, this reduction in contact area will significantly increase plantar contact pressure.[40,42,43] A 25% to 63% reduction in plantar contact area while running in bare feet[44] may counteract the 15% to 33% reduction in impact peak vertical ground reaction forces[9,13] achieved from using a toe-heel-toe strike pattern. This could result in potentially greater contact pressures on the more anterior portions of the metatarsals. High arched runners may experience greater risk of injurious plantar pressures in the lateral metatarsals,[45] while low arched runners may experience greater medial and lateral midfoot contact pressures under a variety of athletic conditions.[46] Concentrating the center of pressure on the midfoot[47] also increases the vertical ground reaction impulse stress (force × time)

on the metatarsals. Previous investigators have reported greater stride frequency with a reduced stride length for individuals who run in bare feet or for individuals who run using a midfoot or forefoot strike pattern.[6,14] Greater peak axial strains and strain rates have been observed in the metatarsals than those in the tibia for barefoot running.[48] Increased stride frequency has been associated with reduced knee and hip loading,[36] however the shorter stride length and increased stride frequency associated with midfoot and forefoot strike patterns will result in more impacts per unit of time and distance, and potentially increased cumulative metatarsal strain compared with rearfoot strike running.

Another potential concern for injury is the increased moment requirement at the ankle joint associated with a more anterior initial foot contact. Runners who use a midfoot or forefoot strike pattern will require greater activation of the plantarflexors during early stance phase to effect the deceleration and then propulsive impulses.[13] This muscular activation may lead to increased mechanical work at the ankle[6,14,49] and additional tensile stress imposed on the plantar flexor muscles and Achilles tendon. Cole et al observed a greater magnitude and rate of loading in the ankle joints during the impact phase of barefoot running compared to shod running.[50]

Supporters of midfoot and forefoot strike running styles blame the initial peak ground reaction force and loading rate associated with a rearfoot strike pattern for increased strains that may injure the lower extremities.[9,31,51] While the initial passive impact peak ground reaction forces that occur at approximately 10% to 12% stance phase are greater for shod heel-toe runners, the midstance active propulsive vertical ground reaction forces may be greater for midfoot or forefoot strikers (Figure 2).[13,14] These greater active propulsive ground reaction forces have not yet been correlated with specific injury risk, but further investigation is warranted.

Figure 2. Vertical ground reaction force curve for the stance phase of gait displaying the initial impact transient (F_{z1}) for shod runners and higher overall propulsive peak ground reaction forces (F_{Zmax}) in midstance for barefoot runners using a midfoot strike pattern. Reprinted from Divert et al[14] with permission.

Few scientific studies have evaluated the POSE running method. Dallam observed a decreased stride length, decreased vertical displacement of the center of mass, and increased oxygen cost when runners used the POSE method compared with traditional heel-toe running in a very small sample.[52] Arendse analyzed 20 individual runners who ran on an outside track, comparing traditional heel-toe running, midfoot strike running after 15 minutes of instruction, and POSE running after 7.5 hours of instruction.[6] Arendse observed decreased stride length and decreased vertical displacement of the center of mass when subjects ran using the POSE method. He reported greater initial vertical ground reaction forces with heel-toe running. Arendse also observed less eccentric angular work at the knee joint and greater eccentric angular work at the ankle joint when subjects ran using the POSE method. This reduction in angular work at the knee joint is often used to promote use of the POSE running method. Reducing knee loading at the cost of increased moment demands at the ankle joint, however, may lead to increased Achilles tendon or other ankle overuse injuries. Fletcher and Romanov also observed reduced stance time, decreased vertical and horizontal displacement of the center of mass, greater knee flexion angular velocity, and greater stride frequency in a sample of 8 runners after 7 hours of POSE running instruction.[53] Again, this increase in stride frequency may result in potentially increased cumulative metatarsal strain and total ankle joint work compared with rearfoot strike running.

The authors were unable to find any peer-reviewed biomechanical analyses of Chi running in the literature. In summary, POSE running, Chi running, barefoot running, and running with a forefoot or midfoot strike pattern have several commonalities. As shown in the Table, these include decreased stride length, decreased vertical displacement of the center of mass, and a possible shift from greater knee joint loading to greater loading at the ankle joint.

Injury Trends

Annual running injury incidence rates have been reported as ranging from 19% to 79%,[2] with the knee joint being the most commonly injured anatomic region among runners.[2,54] We

were unable to identify any previous work separating injury trends by running style. With the majority of modern-day shod runners employing a heel-toe landing style,[3,9] previous injury reports may relate primarily to this running style. Potential causes of running related injuries and various mechanical observations associated with injuries in specific anatomical regions will be addressed. Finally, we discuss injury trends that may be related to wearing traditional running shoes or adopting alternative running styles.

Potential Causes

Many different potential causes have been suggested to explain running injuries. These potential causative factors can be organized into extrinsic and intrinsic factors. Extrinsic factors that may be related to running injury include running shoe age and training errors.[54,55] Training errors may be more associated with injury incidence than biomechanical factors.[55] Exposure to a high training load involving increased intensity, frequency, or running distance[55] without adequate rest may increase the risk of injury,[56] and modification of the training schedule may reduce the incidence of injury.[55-57] The effect of stretching on running injuries has not been determined.[55,58,59]

Intrinsic causes of injury include a previous history of injury,[2,54] increased runner age,[54] increased body mass,[54,60] foot strike characteristics,[23,25,31,61-63] and morphological characteristics such as excessive genu valgum,[64] pes planus,[31] and pes cavus feet.[63] Greater instantaneous and average vertical loading rates have also been observed in runners with a history of injury.[22,65]

Characteristics of various running styles.

	Traditional	Barefoot	Chi	Pose
Stride length	+	–	–	–
Stride frequency	–	+	+	+
Impact transient	+	–	unknown	unknown
Ankle moment	–	+	unknown	+
Knee moment	+	–	unknown	–
Vertical loading rates	+	–	unknown	unknown

+ denotes greater. – denotes lesser.
"unknown" denotes lack of research data.

Ankle and Foot Injuries

Particular characteristics present in subjects with a history of ankle and foot injuries were more years running, weaker plantarflexors, higher arches, and more inversion at touchdown.[59] McCrory et al suggest that plantarflexor insufficiency to control the eccentric phase of dorsiflexion may have contributed to the development of Achilles tendonopathy.[59] A more rigid foot may lead to "compensatory overpronation" that overstresses the Achilles tendon. Reduced tibial external rotation moment and more medial femoral rotation has also been associated with injury in a group of subjects with a history of Achilles tendonopathy.[61] Williams et al propose that this places the lateral gastrocnemius more anteriorly and the medial head of the gastrocnemius more posteriorly.[61] They hypothesized that this shortening of the medial head of the gastrocnemius may have resulted in changes in muscular stress at the musculotendinous junction that may have lead to the development of Achilles tendonitis. Another possible explanation may be that increased internal rotation of the entire lower extremity is associated with increased pronation, which passively stretches the Achilles tendon.

Increased dorsiflexion range of motion and greater instantaneous load rates were observed in a population of females with a previous history of plantar fasciitis.[31] Pohl et al state that the increased passive dorsiflexion range of motion is usually perceived as desirable.[31] They attribute this extra motion to the fact that these previously injured subjects were patients in rehabilitation where they commonly receive plantar flexor stretching exercises as part of their exercise prescription. Since this study was retrospective, the authors were unable to determine if the subjects had the additional range of motion prior to sustaining an injury or if it was acquired during the time the subjects spent in rehabilitation. The authors believe that greater instantaneous rates of loading may subject the plantar fascia to excessive stress.[31] Two groups of investigators have documented that greater pronation and leg length inequality were observed in other samples of plantar fasciitis patients.[63,66] Subotnick previously reported an association between limb length inequality and greater pronation.[67] Warren and Jones also observed greater dorsiflexion and less plantar flexion range of motion in a sample of runners with plantar fasciitis compared to controls.[66] Messier and Pittala observed greater plantar flexion range of motion in their sample of plantar fasciitis patients.[63] They hypothesized that excessive sagittal plane motion may increase the amount of time the runner can impart a propulsive force which may lead to excessive plantar stresses.[63] These authors also attribute greater pronation with greater midfoot stress on the plantar fascia for the injury.[63]

While the balance of running injury literature in the past 30 years assumes a rearfoot strike pattern while wearing traditional shoes, one recent case series detailed 2 marathon runners who sustained metatarsal stress fractures running in barefoot-simulating footwear reportedly adopting a more anterior footstrike.[38] Another recent military study reported reduced incidence of tibial and

femoral stress fractures as the body adapted to the increased military training demands of several cycles of training, but no reduction in metatarsal stress fractures after months of infantry training.[68] This may suggest that the body responds differently to metatarsal stress compared to tibial and femoral stress.

Lower Leg Injuries

Several investigators have examined characteristics of individuals with lower leg injuries. Heel-toe landing styles have been associated with greater anterior compartment pressures than more anterior landing styles.[69] This could be due to a greater activation of the dorsiflexors during initial heel contact compared to a midfoot or forefoot initial contact pattern where greater activation of the plantarflexors has been observed.[13] In a recent case series, 2 previously rearfoot striking patients with chronic exertional compartment syndrome avoided anterior compartment release surgeries by adopting a forefoot striking pattern.[70]

Comparing runners with a history of tibial stress fracture to matched controls, runners with previous tibial stress fractures exhibited greater peak hip adduction and greater rearfoot eversion angles during the stance phase of running.[62,71] Milner and Pohl hypothesized that these forces may have induced a tensile stress on the posteromedial aspect of the tibia.[62,71] These authors also observed greater absolute free moment for individuals who had incurred previous tibial stress fractures.[51,62] Absolute free moment was defined as the torque acting between the foot and the ground at impact which may impose a torsional stress on the tibia.[62] Similarly, greater pronation and velocity of pronation were observed in subjects with a history of shin splints.[63] This increased pronation may increase the stress on the posterior medial tibia as increased stretching of the tibialis posterior imposes greater tensile stress on its proximal attachment site. Greater anterior-posterior braking force and vertical ground reaction forces were observed in another sample of tibial stress fracture patients.[72] Zifchock et al suggest that high peak tibial shock may lead to injury.[72] Creaby and Dixon, however, recently reported no differences in the magnitude of free moment, sagittal, or frontal plane vertical ground reaction forces observed in a small sample of military members with tibial stress fracture compared to matched controls.[32] In a recent systematic review,[73] Zadpoor and Nikooyan contend that greater vertical loading rates and not greater vertical ground reaction forces are more often associated with lower extremity stress fractures.[22,72,74,75] Additionally, no significant intrinsic risk factors were identified in a population of collegiate runners with exercise related leg pain.[76]

Knee Injuries

Multiple intrinsic risk factors have been associated with increased incidence of knee injuries, particularly patellofemoral pain syndrome. Lower extremity malalignment, particularly increased Q-angle and excessive pronation, have been identified as causative factors.[60,64,77,78] Genu valgus changes patellofemoral force vector alignment. Increased body weight and lack of hamstring flexibility may also be related to knee injury.[60] Increased body weight will increase the moment demands on the knee, which will increase the quadriceps and hamstring force production demands. Hamstring tightness may elicit greater knee extension force production, effecting a greater patellofemoral compressive resultant force vector from the knee extensors. Ferber et al recently observed greater peak rearfoot inversion moment, greater peak knee internal rotation angle, and greater peak hip adduction angle in a sample of 35 women with iliotibial band syndrome.[79] Abnormal hip mechanics such as excessive hip internal rotation or adduction possibly due to weakness in the hip abductors may also lead to undesirable knee mechanics and injuries.[80,82] Observed gender differences in strength and alignment may contribute to the running kinematic differences and higher overuse knee injury incidence observed in women.[80-83]

Foot Morphology

In a sample of military recruits, Cowan observed higher odds ratios for lower extremity overuse injuries in soldiers with the highest arches.[84] Messier also observed a similar trend with recreational athletes.[63] Higher arches were associated with greater lower extremity injury incidence in a different sample of female athletes.[85] Higher arches may be associated with rigid feet that do not promote shock absorption at initial foot contact. In a sample of 20 high arched runners, Williams et al observed more bony injuries and lateral injuries in the lower extremities (ie, 5th metatarsal stress fractures, lateral ankle sprains, and iliotibial band syndrome).[86] They also detected more medial injuries, knee injuries, and soft tissue injuries in a sample of 20 low arched runners.[86]

Traditional Running Shoes

In an effort to correct undesirable and possibly injurious mechanics, many healthcare professionals prescribe running shoes with extra cushioning to provide shock absorption, or motion control characteristics to limit pronation.[33,34] Cushioned running shoes may increase contact area and reduce contact pressures in cavus feet.[44,87] Likewise, motion control shoes may increase plantar contact area, reduce tibial internal rotation, and reduce plantar contact pressures in runners with flatter feet.[33,44] Recently, the practice of matching

foot morphology to running shoe type has been questioned.[20,88,89] Even though undesirable mechanics have been prevented in laboratory settings by specific shoe selection and modification, no well-designed studies have demonstrated significant injury reduction by using this commonly used practice of shoe prescription. In the last decade, the results of several studies have demonstrated a correlation between injury and loading rates, and between injury and impact forces.[22,23,31] This growing body of evidence suggests that runners who have developed strike patterns that incorporate relatively low levels of impact forces and a more moderate rate of pronation are at a reduced risk of incurring overuse running injuries. [22,23,31]

Alternative Running Styles

Little to no research exists for injury patterns that may be associated with POSE, Chi, or barefoot running styles. Danny Dreyer claims that the braking forces of heel strike are responsible for many lower extremity overuse injuries.[17-19] The authors could not find any scientific manuscripts in any peer-reviewed journals to substantiate claims that Chi running is safer or superior to traditional heel-toe running mechanics in injury prevention or running economy. Dr Mark Cucuzella presented survey findings of 2500 Chi runners in 2008. Approximately 90% of the runners had favorable impressions of Chi running. Unfortunately, this survey was originally made available to approximately 25,000 people who had purchased Chi running materials and only 10% responded. Theoretically, the adoption of these alternative running styles may shift stress from the knee joint to the ankle joint,[6,8,50] potentially resulting in ankle and foot related injuries. These alternative running forms may be desirable if a runner has a history of knee injuries or knee osteoarthritis and is attempting to shift stress away from the knee joint. More research is needed to compare the mechanics of various running styles and to survey runners who have adopted these running styles for a sufficient period of time to assess the type and severity of injuries they are experiencing.

SUMMARY

Clinicians are often faced with questions from patients about running shoe selection and running style. Traditional shod heel-toe strike running gait has been challenged recently by individuals who advocate a more anterior initial foot contact, or minimal to no footwear which tends to force runners to make initial contact more anteriorly on the foot. Decreasing or eliminating the initial vertical ground reaction peak or "impact transient" has been cited as a potential method to reduce knee joint injuries or injuries in other anatomic regions. This theory requires further investigation to prove its injury prevention claims and to insure that runners who adopt a more anterior strike pattern are not merely increasing their risk for foot and ankle injuries.

Certainly more research is needed to determine ultimately which individuals with certain morphological or mechanical gait characteristics may benefit from alternative running styles that incorporate a more anterior initial foot contact with or without shoes. Controlled longitudinal studies are needed to assess the utility of adopting alternative running styles in an effort to reduce injury rates. Laboratory research comparing the mechanics of various running styles is needed to quantify internal force and moment demands of the various joints in multiple planes. Additional running shoe research is required with large samples of experienced runners to examine the potential effectiveness of matching running shoes to running mechanics and not merely foot morphology. Unbiased injury history surveys are also needed to evaluate the incidence of injury associated with various running styles.

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Clinical and Electrodiagnostic Abnormalities of the Median Nerve in US Army Dental Assistants at the Onset of Training

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ABSTRACT

Purpose/Hypothesis: Dental personnel including dentists, dental hygienists, and dental assistants have been reported as having a high prevalence of upper-extremity musculoskeletal disorders, including carpal tunnel syndrome. Previous research has not involved dental assistant students at the onset of dental training. Therefore, the purpose of this study was to determine the presence of median and ulnar neuropathies in US Army dental assistants at the onset of their training.

Number of Subjects: Fifty-five US Army Soldiers (28 female, 27 male) enrolled in the Dental Assistant (68E) course, volunteered to participate in the study. The mean age of the dental assistant students was 24±7.2 years (range 18-41 years). There were 45 right handed dental assistant students, and the mean length of time in the Army prior to dental training was 27 months (range 3-180 months).

Materials/Methods: Subjects were evaluated during the first week of their 10-week dental assistant course. Subjects completed a history form, were interviewed, and underwent a physical examination. Electrophysiological status of the median and ulnar nerves of both upper extremities was obtained by performing motor and sensory nerve conduction studies. Descriptive statistics for subject demographics and nerve conduction study variables were calculated.

Results: Six of the 55 subjects (11%) presented with abnormal electrophysiologic values suggestive of median mononeuropathy at or distal to the wrist. Five of the subjects had abnormal electrophysiologic values in both hands. Five of these 6 subjects had clinical examination findings consistent with the electrophysiological findings. The ulnar nerve electrophysiologic assessment was normal in all subjects sampled.

Conclusions: The prevalence of median mononeuropathies in this sample of Army dental assistants at the onset of training is greater than 5% prevalence reported in previous healthy populations and is less than 26% prevalence in previous research examining Army dental assistants with dental work experience.

Clinical Relevance: Median neuropathy at or distal to the wrist has been reported in dental personnel including dentists, dental hygienists, and dental assistants, and is also prevalent in this sample of dental assistants at the onset of training. Further long-term prospective research involving the impact of dental practice and techniques for reducing upper extremity injuries in dental professionals appears to be warranted.

There has been a tremendous increase during the past 20 years in the reported cases of carpal tunnel syndrome[1-3] resulting in an increased focus on occupational surveillance and screening.[2] Median mononeuropathy at or distal to the wrist or carpal tunnel syndrome (CTS) is one of a number of muscle-, tendon- and nerve-related disorders that affect people performing intensive work with their hands.[1-3] Dental personnel including dentists, dental hygienists, and dental assistants reportedly have a high prevalence of upper-extremity musculoskeletal disorders, including CTS.[4-16]

In their study investigating the presence of hand problems in US Army dental personnel, Lalumandier et

al[12] stated that 45% of the dental personnel surveyed indicated hand problems, and 25% were determined to indicate a high probability of CTS. The authors concluded that Army dental personnel are at greater risk of developing CTS than the general public.[12] Additionally, Lalumandier et al[12] found that 73% of dental assistants complained of hand problems, and a number of these dental assistants exhibited probable or classic symptoms of CTS. In a separate investigation, Rice et al[13] reported that symptoms associated with CTS were noted by 75.6% of the dental workers, 11% presented diagnosed CTS, and 53% indicated back and shoulder pain. Individuals in the dental assistants group were found to be at risk for developing upper extremity symptoms, CTS and back pain.

Previous studies by our investigative team, which included the diagnostic gold standard of nerve conduction studies, also support an increased prevalence of median neuropathies in military dental personnel. Specifically, 9 (26%) of the 35 Army dental assistants investigated were found to have electrodiagnostic abnormalities of the median nerve at or distal to the wrist (when compared to the ulnar nerve of the same hand).[15] Ulnar nerve electrophysiological function was within normal limits for all subjects examined.[15] This population of dental assistants was studied at the beginning and end of their training to become preventive dental specialists.[15,16] There were no new cases of electrodiagnostic median nerve abnormalities and no statistically significant shift of the nerve conduction values in the prevalence of median mononeuropathies following the 12-week training program.[16] Several ergonomic risk factors are also associated with CTS and include repetitiveness of work, forceful exertions, mechanical stress, posture, temperature, and vibration.[17] These risk factors may be present for dental personnel as dental instruments may cause contact stress over the carpal tunnel, and wrists may be held in awkward positions for prolonged periods.[18]

Collectively, musculoskeletal dysfunction of the upper extremities, to include carpal tunnel syndrome, are well documented in practicing dentists, dental hygienists, and dental assistants. There is also evidence to suggest that short-term dental training does not impact the electrophysiologic status of the median and ulnar nerves.[16] Unfortunately, there is a paucity of information in the literature to document the presence of CTS or upper extremity musculoskeletal dysfunction in Soldiers as they begin their didactic and clinical training program to become dental assistants. The job description for the Army dental assistant is similar to that of a civilian dental assistant. Therefore, the purpose of this study was to determine the presence of clinical and electrodiagnostic abnormalities of the median and ulnar nerves in both upper extremities in this sample of Soldiers at the onset of their training to become dental assistants.

METHODS AND MATERIALS

All Army dental assistants participating in the October through December 2008 dental assistant classes (N=94) were approached to participate in this study. Fifty-five participated, 33 declined, and 6 volunteered but did not keep their appointment for the data collection session.

Experimental procedures, risks, and subject rights were discussed with each individual before participation in the study. All subjects signed an institutionally approved written consent form. Individuals were excluded if they were pregnant. The study was approved by the Institutional Review Board of Brooke Army Medical Center, Fort Sam Houston, Texas.

A history, physical examination, and upper quarter neuromusculoskeletal screen were performed to determine the musculoskeletal status of the neck and upper extremities, and neural integrity of the median and ulnar nerves. These assessments were performed during the first week of the dental assistant training program.

History

A history was taken from each patient in questionnaire format. The history included information pertaining to demographics, medical history, military background, work experience, hand dominance, and the amount of time using a computer.

Physical Examination

As part of the evaluation process, the physical (screening) examination of each participant included assessment of active range of motion, manual muscle tests, sensory evaluation, reflex testing, and select special tests.[19,20] Specifically, active range of motion was assessed for the cervical spine, shoulders, elbows, wrists, and hands. Manual muscle testing was performed for all major muscle groups in both upper extremities. Sensory assessment was determined with light touch, vibration, and pain/pin prick assessment of the bilateral upper extremities. Light touch and pin prick sensation included the bilateral C4-T1 dermatomes. Vibration sensory testing was conducted using the Biothesiometer (Biomedical, Newbury, Ohio). Testing was performed at the dorsal 1st metacarpal and distal tip of the thumbs, long fingers, and middle fingers. Muscle stretch reflexes (also known as deep tendon reflexes) were obtained from the biceps brachii, brachioradialis, and triceps in the upper extremities. Upper extremity pathological reflexes were assessed with the Hoffman sign.

Last, the special tests of Tinel's sign of median and ulnar nerves at the wrist, Tinel's sign of the ulnar nerve at the elbow, Phalen's test, and the assessment of the radial pulses during positional changes of the upper extremities and neck (Adson's maneuver) were examined.[19,20] Additional special tests of the median and ulnar nerves were performed on both upper extremities of each subject and included the elbow hyperflexion test (EHFT) to assess for ulnar neuropathy at the elbow,[21] upper limb neural dynamic testing (ULNDT) to determine irritation of the cervical nerve roots or upper extremity nerves,[22] and wrist ratio tests that determines the ratio of both the anterior-posterior and medial-lateral widths measured at the distal crease of the wrist.[23-25]

Nerve Conduction Studies

At the time of volunteer solicitation, potential subjects were instructed to abstain from exercising for 1 hour prior to testing. Skin temperature at the wrist was measured using a digital thermometer model TM99A (Cooper Instrument Corporation, Middlefield, Connecticut), and was maintained at or above 32ºC. If skin temperature fell below this value, the wrist, hand, and forearm were rewarmed with warm towels.

The Cadwell Sierra LT electromyograph and stimulator (Cadwell Laboratories, Inc, Kennewick Washington) were used to measure the compound motor action potential (CMAP) and sensory nerve action potential (SNAP) latencies and amplitudes. The stimulating current was a monophasic pulse 0.1 millisecond long. The oscilloscope was set to a sweep duration of 2.0 milliseconds per division and a gain of 20 μV per division for the SNAPs. For the CMAPs, the oscilloscope was set to a sweep duration of 2.0 milliseconds per division and a gain of 5 mV per division. The filter settings were 10 Hz-10 kHz for the motor potentials and 10 Hz-2 kHz for the sensory potentials. The sensory latency was measured at the negative peak of the SNAP, and the amplitude was measured from negative peak to positive peak. The motor latency was measured from the negative takeoff of the evoked CMAP, while the amplitude was measured from the baseline to the negative peak of the evoked response. The obtained results were recorded manually and on computer printout.

Specific details for performing the median and ulnar nerve conduction studies (NCS) were presented in a study by Harkins et al[26] and follow procedures previously described.[15,27-30] The median and ulnar nerve palmar and digital distal sensory latencies (DSLs), distal motor latencies (DMLs), and conduction velocities were obtained from both upper extremities. All NCS procedures included measuring the anatomic course of the nerve: median and ulnar palmar DSLs (8 cm), median and ulnar digital DSLs (14 cm), and median and ulnar DMLs (8 cm). In addition to comparing median nerve palmar and digital DSLs, DMLs, and conduction velocities with a chart of normal values, comparison studies between median and ulnar palmar DSLs, digit DSLs (digit 2 and digit 5, digit 4 median/ulnar), and DMLs in the same and opposite extremities were obtained. Examination of median and ulnar latencies in the same extremity and median and ulnar latencies in opposite extremities has been shown to assist in early electrodiagnosis of CTS.[27-30]

Preventive Guidelines and Exercises for the Wrist and Hand

Upon completion of the examination, each Soldier received information regarding stretching, strengthening, resting, and nerve gliding techniques to assist in the prevention of future musculoskeletal injury. The exercises focused on the forearm, wrist, and hand. The purpose of providing upper extremity exercises (mobility, strengthening, and stretching) was to increase these Soldiers' awareness of possible musculoskeletal problems in dental personnel. Additionally, the subjects were advised to incorporate these exercises into their professional dental assistant practice.

In an effort to ensure consistency across subjects, one investigator (N.H.) collected all history data, another (S.F.) performed the physical examinations, and a third (R.M.) performed all nerve conduction tests. The neural conduction assessment of the median and ulnar nerves that were performed by researcher R.M. was directly monitored by either investigator 4 (D.G.), 5 (S.S.), or 6 (J.M.). Investigators 4 and 5 are board-certified clinical specialists in clinical electrophysiology by the American Board of Physical Therapy Specialties of the American Physical Therapy Association. Investigators 4, 5, and 6 are or have been credentialed by the US Army to perform clinical electrophysiological testing (NCS and EMG studies).

Data Management and Analysis

Descriptive statistics for subject demographics and nerve conduction study variables were calculated using Statistical Package for Social Sciences (SPSS) software version 12.0 (SPSS Inc, Chicago, IL).

RESULTS

History

Fifty-five subjects participated in this study (28 female, 27 male). The age of the subjects ranged from 18 to 45 years (mean=24±7 years). Forty-five of the subjects were right-handed. All subjects had been in the military for at least 3 months with a range of 3 to 180 months

(mean=27 months), and had completed basic combat training. Three of the subjects had college degrees, 19 had completed some college work, 31 had a high school diploma, and 2 had a GED. Twenty-three subjects were active duty Soldiers, 19 Army Reservists, and 13 Army National Guardsmen. One subject (No. 49) had previous experience as a dental assistant in a civilian setting.

The mean time the subjects spent working on a computer was 5 hours per week with a range of 0 to 40 hours per week. Fifteen of the subjects played some type of musical instrument including guitar, violin, clarinet, flute, piano, and drums. The subjects participated in sporting activities an average of 5 hours per week with a range of 0 to 30 hours.

When asked to describe their general health, 46 of the subjects reported being in excellent/good health and 9 of the subjects reported fair health. None of the subjects reported having a history of neuropathic disease, renal disease, peripheral vascular disease, thyroid disease, or diabetes. Two subjects reported a history of having arthritis. Fifteen of the subjects responded positively to having a problem with their head, neck, or upper extremities during the previous 6 months prior to data collection. All other subjects denied having any musculoskeletal problems in the past 6 months.

The following information was received in response to specific questions about current pain or symptoms in the neck or upper extremity: dull, aching neck pain and in the C7-T1 dermatomes (subject 8); cramps in the left hand (subject 26); dull, achy pain in the left D3-D4 fingers (subject 37); and numbness and tingling in both hands (subject 46).

A review of the subjects' histories revealed that subjects 8, 37, and 46 had subjective complaints suggestive of median or ulnar dysfunction. Otherwise, there were no indicators in the information obtained in the history suggesting median or ulnar nerve abnormalities in the upper extremities of these dental assistants.

Screening Examination

Active Range of Motion: Two subjects had limited active range of motion (AROM). One had limited right shoulder internal rotation and one had limited right elbow extension and forearm supination. All other subjects had normal AROM of the cervical spine, shoulder, elbow, wrist, and hands bilaterally. Cervical quadrant tests were determined to be normal without radicular symptoms in either upper extremity.

Motor Strength: All subjects were assessed to have normal (5/5) muscle strength for the neck flexors, extensors, and rotators (C1-5); both upper extremities to include the scapula elevators, depressors, protractors, and retractors (C1-5); shoulder flexors, abductors, and external/internal rotators (C5-6); elbow flexors and extensors (C5-8); wrist flexors and extensors (C6-8); finger flexors and extensors (C7-T1); and hand intrinsics (C8-T1). All upper extremity peripheral nerves (motor components) and myotomes (C4-T1) were assessed during motor testing.

Sensation: Five subjects had abnormal sensation to light touch. Sixteen subjects had abnormal palm to tip light touch sensation. Eight subjects had abnormal vibration sensation (3 metacarpal, 3 thumb, 1 long finger, 1 little finger). Of the subjects with abnormal sensation, only 3 subjects had abnormal median nerve findings on electrophysiological testing (subject 37, light touch and pain; subject 49, palm to tip light touch; subject 20, abnormal vibration test). Otherwise, all subjects had normal peripheral nerves (sensory components) and C4-T1 dermatome sensory testing results for light touch, pain/pin prick, and vibration in both upper extremities.

Muscle Stretch Reflexes (MSRs) and Pathological Signs: Two subjects had absent MSRs. One subject had absent brachioradialis (C6) MSRs bilaterally, and a second subject had an absent left brachioradialis (C6) MSR. Otherwise, all subjects displayed present and equal muscle stretch reflexes. Pathological signs (Hoffman) were absent in both upper extremities of all subjects.

Special Tests: Twelve subjects had positive Tinel's tests (3 median nerve at the wrist; 1 ulnar nerve at the wrist; 8 ulnar nerve at the elbow). Only one subject (No. 26) had a positive Tinel's sign of the median nerve at the wrist and abnormal electrophysiological findings in the median nerve. Three subjects had positive Phalen's test for the median nerve. One subject had a positive EHFT. None of the subjects with a positive Phalen's test for the median nerve or EHFT for the ulnar nerve had abnormal electrophysiologic findings on NCS testing. Twenty subjects had positive ULNDTs for the median nerve. Only subject 37 had a positive ULNDT for the median nerve and abnormal electrophysiologic findings on NCS testing. Forty-two subjects (76.1%) had wrist ratios (WR) $\geq$0.70. Four subjects (8, 20, 26, 37) with abnormal WR also had abnormal median nerve electrophysiological findings.

Thoracic Outlet Tests: All subjects displayed normal radial pulses when the upper extremities were tested in the 3 thoracic outlet syndrome testing positions.[19,20]

Other than 5 subjects (8, 20, 26, 37, 49) who had abnormal findings on physical examination and abnormal median nerve electrophysiologic testing, there were no diagnostic indicators or evidence of median and ulnar nerve abnormalities in either upper extremity of the dental assistants tested during the physical examination portion of the assessment.

Table 1. Mean, standard deviation, and range of values for neural conduction measurements.

	Right Upper Extremity			Left Upper Extremity			Normal Values
Median Nerve	Mean	SD	Range	Mean	SD	Range	
Motor							
DML (milliseconds)	3.5	0.4	2.8-4.6	3.5	0.5	2.7-5.5	<4.2
Amp CMAP (mV)	9.0	2.2	5-16	8.7	2.9	5.0-17	>5
MNCV BE - W(m/sec)	59	4.1	50-67	59	4.1	51-67	>50
Sensory							
Palmar DSL (ms)	1.9	0.2	1.6-2.4	1.9	0.2	1.5-2.9	<2.2
Palmar Amp SNAP (μV)	117	41.9	20-200	103	41.8	23-200	>15
2 Digit DSL (milliseconds)	3.0	0.2	2.5-3.6	2.9	0.3	2.5-4.0	<3.5
2 Digit Amp SNAP (μV)	31	12	15-66	28	10.5	16-55	>15
Ulnar Nerve							
Motor							
DML (milliseconds)	2.8	0.3	2.2-3.4	2.8	0.3	2.3-3.3	<3.6
Amp CMAP (mV)	9	2.5	5-17	8	2.3	5-14	>5
MNCV BE - W (m/sec)	64	4.1	54-73	62	4.4	51-74	>50
MNCV AE - BE (m/sec)	67	5.6	54-77	65	6.2	52-76	>50
Sensory							
Palmar DSL (milliseconds)	1.7	0.1	1.5-2.1	1.7	0.1	1.5-2.1	<2.2
Palmar Amp SNAP (μV)	44	27.0	15-153	48	26.8	16-130	>10
5 Digit DSL (milliseconds)	2.8	0.2	2.5-3.5	2.9	0.2	2.5-3.2	<3.5
5 Digit Amp SNAP (μV)	23	6.8	14-52	26	10.8	12-79	>10

Glossary
AE – above elbow
Amp – amplitude
BE – below elbow
CMAP – compound motor action potential
DML – distal motor latency
DSL – distal sensory latency
MNCV – motor nerve conduction velocity
SNAP – sensory nerve action potential
W – wrist

Nerve Conduction Studies

The results of the nerve conduction studies are presented in Table 1. The values for these electrophysiological variables for each subject were compared to a chart of normal values (Table 1). This chart of normal values was developed in the Clinical Electrophysiological Laboratory of Texas Physical Therapy Specialists (New Braunfels, Texas), and revalidated at the Electrophysiological Laboratory, Army-Baylor University Doctoral Program in Physical Therapy. The chart of normal values depicted in Table 1 is similar to other charts of normal values.[27-30]

A comparison of the results of the study with the chart of normal values determined that 4 subjects (8, 20, 37, 46) had electrophysiologic evidence of median mononeuropathy at or distal to the wrist (Table 2).

Interestingly, when comparison studies between the median and ulnar palmar DSLs, digital DSLs, and DMLs in the same and opposite extremities, and the digit 4 median/ulnar comparison study in the same extremity were assessed, electrodiagnostic abnormalities of the median nerve at or distal to the wrist in an additional 2 subjects (26, 49) were revealed (Table 2).

Six of the 55 subjects (11%) presented with abnormal electrophysiologic values suggestive of median mononeuropathy at or distal to the wrist. Five of the subjects had abnormal electrophysiologic values in both hands. Three of the subjects (8, 20, 37) had electrophysiologic abnormalities of both the motor and sensory fibers , and three (26, 46, 49) had abnormal findings in only the sensory fibers (Table 2). Five of the 6 subjects had clinical examination findings consistent with the electrophysiological findings. (Tables 3 and 4) The ulnar nerve electrophysiologic assessment was normal in all subjects.

COMMENT

To our knowledge, no studies have used histories, physical examinations, and NCS to assess the status of the median and ulnar nerves in Soldiers enrolled in a dental assistant course. A thorough history and physical examination are considered essential screening tools for detecting signs and symptoms of peripheral neuropathy.[28-30,32-34] Nerve conduction measurement is often performed on the median and ulnar nerves to determine whether certain entrapment neuropathies are present.[28-42] Nerve conduction measurement is considered the gold standard when assessing the electrophysiologic status of the peripheral nerve.[28-42]

Of the 55 Soldiers in the dental assistant course that participated in the study, 11 hands (10%) in 6 subjects (11%) had electrodiagnostic abnormalities of the median nerve at or distal to the wrist. Subject 8 was an 18-year-old right hand dominant (RHD) male who worked in

the fast food industry prior to joining the military. He regularly played the guitar 12 hours/week and worked on the computer for 21 hours/week. The subject complained of a dull, aching pain in the bilateral C7-T1 dermatome but had no other symptoms suggestive of median mononeuropathy at or distal to the wrist. Physical examination determined that the patient's bilateral wrist ratios were >0.70, but otherwise there were no findings on the physical examination suggestive of a median mononeuropathy. During NCS testing, subject 8 had bilateral median mononeuropathy at or distal to the wrist that affected both the motor and sensory fibers.

Subject 20 was a 45-year-old RHD male who had been a mail carrier before entering the military and had been worked in the military for 8 years prior to the dental assistant course as an infantryman, mechanic, and vehicle operator. He had no symptoms including pain, numbness/tingling, or weakness in bilateral upper extremities (BUE). However on physical examination, the subject displayed an abnormal left wrist ratio (>0.70), decreased vibration sensation on the right metacarpal area, and decreased vibration sensation on the left D1-D2-D3. He had bilateral median mononeuropathy at or distal to the wrist affecting both motor and sensory fibers on the left and motor fibers only on the right.

Table 2. Subjects with positive findings on neural conduction comparison studies.

		Palmar DSL*			Digital DSL*		
Subject	Hand	Median	Ulnar	Difference	Median	Ulnar	Difference
8	R	2.3	1.7	0.6	3.4	2.9	0.5
	L	2.3	1.7	0.6	3.4	3.0	0.4
20	R	2.2	1.6	0.6	3.2	2.7	0.5
	L	2.3	1.7	0.6	3.4	2.8	0.6
26	R	2.2	1.6	0.6	3.4	2.8	0.6
	L	1.9	1.6	0.3	3.1	2.8	0.3
37	R	2.4	1.9	0.5	3.5	2.9	0.6
	L	2.9	1.8	1.1	4.0	2.9	1.1
46	R	2.4	1.6	0.8	3.6	2.9	0.7
	L	2.2	1.8	0.4	3.3	2.7	0.6
49	R	2.1	1.6	0.5	3.5	2.9	0.6
	L	1.9	1.7	0.2	3.0	3.0	0.0
		DML*			D4 DSL*		
Subject	Hand	Median	Ulnar	Difference	Median	Ulnar	Difference
8	R	4.6	2.8	1.8	3.6	2.8	0.8
	L	4.5	2.9	1.6	3.4	3.3	0.1
20	R	4.3	2.7	1.6	3.3	2.9	0.4
	L	4.3	2.7	1.6	3.6	2.6	1.0
26	R	3.7	2.7	1.0	3.3	2.8	0.5
	L	3.5	2.9	0.6	3.4	2.7	0.7
37	R	4.3	2.8	1.5	3.6	3.1	0.5
	L	5.5	2.9	2.6	4.3	3.1	1.2
46	R	4.2	2.6	1.6	3.6	2.9	0.7
	L	3.8	2.6	1.2	3.5	2.7	0.8
49	R	4.2	2.5	1.7	3.3	2.9	0.4
	L	3.6	2.6	1.0	3.0	2.8	0.2

*All units are milliseconds.

DSL indicates distal sensory latency. DML indicates distal motor latency.

Notes:

Prolonged DSL (palmar and digit) difference ≥0.6 (normal ≤0.5)
Prolonged D4 median/ulnar difference ≥0.7 (normal ≤0.6)
Prolonged median D2 DSL ≥3.7 (normal ≤3.6)
Prolonged DML difference ≥1.1 (normal ≤1.0)
Prolonged median palmar DSL ≥2.3 (normal ≤2.2)
Prolonged DML ≥4.3 (normal ≤4.2)

Subject 26 was a 21-year-old left hand dominant (LHD) female who had a 3-month history of bilateral hand cramping and a family history of arthritis and CTS. The subject did not list any previous occupations before entering the military 3 months prior to the testing session. Physical examination determined that this subject had an abnormal bilateral wrist ratio (>0.70), and a positive Tinel's sign over the right median nerve at the wrist. The subject had early bilateral median mononeuropathy at or distal to the wrist of the sensory fibers based on comparison studies. Subject 26 also had a positive Tinel's sign of the right ulnar nerve at the wrist, but NCS studies of the right ulnar nerve were normal.

Subject 37 was a 39-year-old LHD male who has been a medic in the Army National Guard for 13 years. Prior to entering the military, subject 37 had been an ambulance and rescue worker. Subject 37 had a 6-month history of a dull, aching pain in the left D3-D4. Physical examination revealed an abnormal bilateral wrist ratio (>0.70), abnormal sensation to light touch and pinprick in the left C6 dermatome, and an abnormal bilateral ULNDT examination. The subject had bilateral median mononeuropathy at or distal to the wrist affecting both motor and sensory fibers. The subject had normal NCS studies of both ulnar nerves.

Subject 46 was a 29-year-old RHD male who had no current symptoms in BUE but stated occasional numbness and tingling in both hands prior to joining the military 6 months before the exam. In civilian life, the subject was an electrician and played the guitar approximately one hour per week. He had no findings on physical examination to suggest a bilateral median mononeuropathy at or

Table 3. Symptoms and history of subjects with electrophysiologic evidence of median mononeuropathy at or distal to the wrist.

Subject (+NCS)	Symptoms	Duration of Symptoms	Patient History
8 (R/L)	Dull, achy pain in C7-T1 dermatome bilaterally	Unknown	Guitar player, worked in fast food industry
20 (R/L)	None	N/A	Mail carrier; previous military: infantry, mechanic, and vehicle operator
26 (R/L)	Cramps in left hand intrinsics	3 months	Family history of CTS and arthritis
37 (R/L)	Dull, achy pain in left middle/ring fingers	6 months	Ambulance and rescue worker
46 (R/L)	No current symptoms; N/T in hands prior to joining military	N/A	Family history of CTS; previous work history as electrician, guitar player
49 (R)	None	N/A	Dental assistant; waiter

NCS indicates nerve conduction studies. CTS indicates carpal tunnel syndrome.

distal to the wrist. Subject 46 had a right median mononeuropathy at or distal to the wrist affecting the sensory fibers (prolonged palmar DSL), and an early left median mononeuropathy affecting the sensory fibers based on comparison studies.

Subject 49 was an 18-year-old RHD female and was the only soldier in the study that had prior experience and work history (2 years) as a civilian dental assistant. She had no symptoms in BUE. On physical examination, she had no findings suggestive of median mononeuropathy, but had abnormal sensation to light touch of the right D5 and a positive Tinel's sign of the right ulnar nerve at the elbow. NCS studies of the right ulnar nerve were normal. On NCS testing, subject 49 had an early right median mononeuropathy at or distal to the wrist affecting only the sensory fibers based on comparison studies.

Four of the 6 subjects with abnormal electrophysiological findings of median mononeuropathy at or distal to the wrist had a wrist ratio ≥0.70. It is important to note that 42 subjects (76%) had an abnormal wrist ratio ≥0.70, but only 3 subjects had clinical electrophysiologic evidence of median mononeuropathy at or distal to the wrist and abnormal wrist ratios. There is growing evidence suggesting that wrist anatomy may predispose individuals to carpal tunnel syndrome.[23-25] Specifically, 3 separate studies have identified that "square-shaped" wrists, or wrists with larger wrist ratio indexes (>0.67-0.70), appear to be predisposed to CTS.[23-25] To obtain the ratio both the anteroposterior (AP) and mediolateral (ML) widths are measured at the distal crease of the wrist. The wrist ratio is then calculated by dividing the AP width by the ML width. A 2005 study revealed that this measure was both reliable and sensitive for determining the presence of electrodiagnostically confirmed CTS.[24] Other than the wrist ratio, there were no other consistent physical exam findings across subjects in this study.

In this population of Soldiers entering the dental assistant training program, 5 of the 6 subjects with electrophysiologic evidence of median mononeuropathy at or distal to the wrist had prior occupations both in the civilian and military workforce. Subject 8 worked in the fast food industry; subject 20 was a mail carrier (civilian) and an infantryman, mechanic, and vehicle operator in the military; subject 37 worked as an ambulance and rescue worker; subject 46 was an electrician; and subject 49 was the only subject in this population with experience as a civilian dental assistant. It appears that these subjects with electrophysiologic evidence of median mononeuropathy at or distal to the wrist may indeed be pre-exposed to disorders that affect people performing intensive work with their hands.[1-3]

Three of the subjects (8, 46, 49) had known risk factors for developing median mononeuropathy at or distal to the wrist. Subject 49 had previous civilian dental experience. Median mononeuropathies at or distal to the wrist have previously been reported in the dental assistant population.[12,13,15,16] Two subjects (8, 46) played musical instruments (guitar), and there is evidence to support that guitarists are susceptible to having median mononeuropathies at or distal to the wrist.[43] It is interesting to note that if we exclude the 3 subjects with known risk

Table 4. Physical examination findings on subjects with electyrophysiologic evidence of median monomeuropathy at or distal to the wrist.

Subject	Dominant Hand	+NCS	Physical Exam Findings
8	R	B	R&L wrist ratio ≥ 0.70
20	R	B	Left wrist ratio ≥ 0.70; right metacarpal abnormal vibration; left metacarpal/thumb/long & little finger abnormal vibration
26	L	B	R&L wrist ratio ≥ 0.70; right abnormal ROM; right Tinel's median nerve at wrist & ulnar nerve at wrist
37	L	B	R&L wrist ratio ≥ 0.70; abnormal sensation left C6 dermatome; R&L ULNDT
46	R	B	no positive findings
49	R	R	Right abnormal palm-to-little-fingertip light touch; right Tinel's ulnar nerve at elbow

NCS indicates nerve conduction studies. R&L indicates right and left. ULNDT indicates upper limb neural dynamic testing.

factors, the prevalence of median mononeuropathy at or distal to the wrist is reduced to 5.45%, which is consistent with previous reports on the general population.[44]

In a population such as the Soldiers enrolled in a dental assistant training program that were studied here, it is reasonable to expect that subclinical upper extremity mononeuropathies secondary to repetitive overuse may be present. In the early stages of a mononeuropathy of this type, many individuals with a clinically detectable problem are not aware that their neural function has been impaired.[44] Atroshi et al[44] examined 125 asymptomatic controls with NCS of the median nerves and they reported that 18% (n=23) had electrophysiological evidence of carpal tunnel syndrome.

Atroshi et al[44] stated that the estimation of the prevalence of carpal tunnel syndrome in a general population may contribute to the early diagnosis and effective treatment of subjects and provide useful data for the interpretation of results that estimate the prevalence of carpal tunnel syndrome in specific occupational groups. Franzblau and Werner[45-47] further suggest that performing NCS on individuals without symptoms of carpal tunnel syndrome is important because it permits the assessment of the overall relationship between the electrophysiological properties of the nerve and other clinical features of carpal tunnel syndrome. Although no strong evidence exists regarding the prevention or progression of CTS, it makes sense, theoretically, to identify a problem early, where a minor intervention, such as a resting night splint or ergonomic changes in the work environment, might rectify the dysfunction.[45-47]

A valuable extension of this study would be evaluation of these dental assistants as they progress through their dental health careers and to reevaluate these dental assistants in 5 to 10 years to determine whether these individuals who presented with early electrodiagnostic abnormalities of median nerve at or distal to the wrist later develop symptomatic carpal tunnel syndrome. Future research should include the development of more accurate diagnostic tests performed during the physical examination to determine the presence of median mononeuropathy at or distal to the wrist. Since the majority of current evidence is based on individuals already being symptomatic, a larger study of the general population would be required to more accurately determine prevalence. Last, a longitudinal study should be conducted to determine if there is a relationship of physical examination findings as predictors for development of CTS. This could be developed into an occupational screening tool that would identify individuals at greater risk. Additional long-term prospective studies examining the prevalence and prevention of upper extremity disorders, to include carpal tunnel, appear to warrant further investigation.

CONCLUSION

This descriptive study examined a sample of 55 US Army Soldiers who were enrolled in a training program to become dental assistants for the presence of median and ulnar neuropathies. Six of the 55 subjects (11%) presented with abnormal electrophysiologic values suggestive of median mononeuropathy at or distal to the wrist. Five of the subjects had abnormal electrophysiologic values in both hands. Five of these 6 subjects had clinical examination findings consistent with the electrophysiological findings. The ulnar nerve electrophysiologic assessment was normal in all subjects sampled.

The prevalence of median mononeuropathies in this sample of Soldiers at the onset of training to become dental assistants is greater than 5% prevalence reported in previous healthy populations and is less than 26% prevalence in previous research examining Army dental assistants with dental work experience. These findings suggest that nerve conduction comparison studies may provide sensitive measures and early indicators for detecting early median nerve compromise at or distal to the wrist. Additional prospective research is required to validate our findings and determine the factors that are predictive of activity limitations and participation restrictions in dental assistants who develop carpal tunnel syndrome.

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Erratum

In the article "Reliability of Lower Quarter Physical Performance Measure in Healthy Service Members" published on pages 37-49 of the July-September 2011 issue of the *AMEDD Journal*, the byline entry "John C. Childs" is incorrect. The correct byline entry is "John D. Childs."

Risky Business: Challenges and Successes in Military Radiation Risk Communication

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ABSTRACT

Given the general public's overall lack of knowledge about radiation and their heightened fear of its harmful effects, effective communication of radiation risks is often difficult. This is especially true when it comes to communicating the radiation risks stemming from military operations. Part of this difficulty stems from a lingering distrust of the military that harkens back to the controversy surrounding Veteran exposures to Agent Orange during the Vietnam War along with the often classified nature of many military operations. Additionally, there are unique military exposure scenarios, such as the use of nuclear weapons and combat use of depleted uranium as antiarmor munitions that are not found in the civilian sector. Also, the large, diverse nature of the military makes consistent risk communication across the vast and widespread organization very difficult. This manuscript highlights and discusses both the common and the distinctive challenges of effectively communicating military radiation risks, to include communicating through the media. The paper also introduces the Army's Health Risk Communication Program and its role in assisting in effective risk communication efforts. The authors draw on their extensive collective experience to share 3 risk communication success stories that were accomplished through the innovative use of a matrixed, team approach that combines both health physics and risk communication expertise.

RISK COMMUNICATION: WHAT IT IS AND WHAT IT IS NOT

Chances are, if you asked a group of health physicists to define risk communication, you would get a wide variety of answers. Fortunately, there is a commonly accepted definition of risk communication:

> Risk communication is an interactive process of the exchange of information and opinion among individuals, groups, and institutions. It involves multiple messages about the nature of risk and other messages, not strictly about risk, that express concerns, opinions, or reactions to risk messages or to legal and institutional arrangements for risk management.[1]

Although communication with the public is typically thought to be a public affairs or public relations function, the authors view risk communications as a unique discipline with expertise in communication that combines an understanding of science and its limitations with an appreciation of the psychology and sociology of how people, individually and collectively, process, understand, and ultimately come to accept or reject risks to human health.

Having provided a commonly accepted definition of risk communication, we now attempt to debunk some popular myths about it. First, risk communication is not a "quick fix" for dealing with a crisis, nor is it an afterthought in responding to an emergency, or a panacea for handling public concerns. Risk communication is never a one-way dialogue simply "telling" the public what the risks are, thereby ending the matter. Nor is risk communication public affairs or public information, where the purpose is to convey an organization's message, story, or agenda.[1] And finally, risk communication is never, ever "spin." In its truest sense, risk communication is a combination of "tools" to be used when concern is high, and "processes" that integrate risk communication factors into the overall risk management of an issue. This paper outlines the use of these concepts in actual real-world situations involving radiation risk.

COMMON AND UNIQUE CHALLENGES OF MILITARY RADIATION RISK COMMUNICATION

Communicators of military radiation risk share all of the common challenges of anyone conveying radiation risks. First, the very nature of radiation makes communicating its risks very difficult. Although radiation is ubiquitous, exposure is imperceptible to the human senses, making it both unfamiliar and seemingly nefarious. Also, radiation risk is highly complex: radiation exposure at very high doses can cause immediate death clearly due to its effects, while at low doses it may or may not cause cancer years or decades after exposure (and if induced, these cancers cannot be identified as radiogenic). Further complicating matters is the fact that radiation can be both an internal and external hazard, depending upon the radionuclide and the type of radiation it emits (alpha, beta, gamma, etc). The general public's overall understanding about radiation is also extremely limited and often tainted and distorted by misrepresentation of

its risks in popular culture, the news media, and by activists. Finally, society's risk appetite has changed over time, with increased demands by society's members to be involved in risk management decisions that personally affect them, and a decreased overall societal tolerance of risk in general (eg, demands for zero risk).

Added to this already contentious situation are the unique challenges of communicating military radiation risks. First, there is a latent distrust of the military that harkens back to the legacy of veteran exposures to Agent Orange during the Vietnam War. Also, many military operations are classified, thereby serving as a serious barrier to open risk communication. To further complicate things, the military also has its own unique and sometimes unfamiliar radiation sources, such as nuclear weapons and depleted uranium which, because of its unique metallurgic properties, makes it both an ideal antiarmor munition and armor plating. Finally, the military is a large, diverse, bureaucratic organization with many stovepipe* and silo† components, making consistent risk communication a constant challenge.

COMPONENTS OF RISK COMMUNICATION

In the field of general communication, there are 3 distinct components, normally listed in this order: messenger, message, and audience. However, in health physics, one typically starts with the message (what is the dose), then focuses on the audience (patients, workers, general public), and rarely, if at all, do health physicists think about themselves, the messengers. Conversely, in this paper, we deliberately choose to begin with the audience because, in our opinion, understanding the audience is the most important part of effective risk communication. However, as mentioned previously, it is usually considered secondarily, at best. Important information about the audience includes their actual concerns (not what the experts think are their concerns); other risks they may be facing; their level of understanding of science and their trust in both it and in scientists; their preconceptions about radiation and its risks; and other cofactors such as possible economic loss due to radiological contamination, potential stigma by being "contaminated or exposed," and their overall perception of social justice. Research also shows that the human brain processes risk information differently when concern is high, so it is important to account and plan for these changes in message development and overall risk communication efforts, particularly about radiation risks.[4]

*An organizational structure in which the flow of information is restricted to up and down through lines of control but is inhibited or prevented from moving across the organization.[2]

†A silo structure is one that functions almost entirely within itself, without interaction, communication, or cooperation with other components of the organization.[3]

Next, we address the messenger, the one actually communicating the risk. Often, health physicists find themselves as risk communicators because of their unique expertise in radiation safety. However, while this expertise is essential, the most important trait in an effective risk communicator is empathy.[5] This is because worried people need their emotions and perceptions about specific risks verbally and visibly acknowledged by the risk communicator before productive communication can take place. Additionally, a messenger must be open, honest, and sincere. Since many risk communication events can be quite emotionally heated, the health physicist must also be able to practice the fine art of deflection and detachment, not taking any anger or hostility personally (which sounds easy but is very difficult to do in practice!). Other factors to consider are the ability to deal with uncertainty by describing what is known, what is not known, and what will be done to fill any data gaps; a genuine commitment to follow up; and being both willing and prepared to go the extra distance to address the audience's concerns (such as offering dosimetric monitoring or bioassay sampling even when it is not legally required or deemed scientifically necessary).

Finally, we address the message. Although it's tempting to merely develop messages based on a radiological assessment alone, the most effective messages are those that balance what the audience wants to know with what you need to provide (thus our focus on the audience first). No more than 3 messages should be provided in a given situation, since the human brain when under stress is capable of processing only limited amounts of information.[6] Messages should be simple (provided in the language of the audience) and concise, but not condescending. Also, messages should always avoid the use of jargon and never include humor. The messages should be brief (7-12 words, if possible) and include the reemphasis of its clear points. Whenever possible, messages should be validated by credible independent third party sources, such as the National Council on Radiation Protection and Measurements or the International Commission on Radiological Protection for international audiences.

THE ARMY'S HEALTH RISK COMMUNICATION PROGRAM

Health risk communication expertise within Army Medicine is available from 2 sources. First, risk communication expertise is now available within the Communication Directorate at the Army Medical Command (MEDCOM) headquarters. This is a new skill set within the Directorate intended to support issues MEDCOM-wide and is slowly being integrated into sensitive, high-profile projects throughout the Command. The subject matter expert (SME) provides senior-level risk communication

guidance to identify and develop strategies to minimize communication and reputational risks, strengthen audience confidence in Army medicine, increase risk and crisis communication skills level and standardize crisis communication response throughout MEDCOM, and improve the effectiveness of communication efforts. The SME has provided risk communication recommendations and guidance to numerous MEDCOM-wide issues, including the temporary removal of dietary supplements, Soldier death from rabies, allegations of inadequate behavioral health care, use of expired blood products, and allegations of the use of recalled test questions in the Army's radiology residency program. The risk communication SME has also provided onsite assistance to medical risk communication issues, such as the recent medical reevaluations of Soldiers seen by the forensic psychiatry team at the Madigan Army Medical Center.

The second source is the Army Public Health Command's Health Risk Communication Program (HRCP), established in 1989 in response to increasing demands from the Army and the public for a broader approach to public health risks. The HRCP initially focused on risk communication training, but the program has expanded and now provides technical consultative expertise to customers throughout the Department of Defense, responding to the broad spectrum of health risk communication issues, including radiation. The HRCP staff members are highly trained and seasoned health risk communicators with diverse academic backgrounds, including education, public health, and health communication.

The HRCP supports the 3 components of the risk communication process (audience, messenger, and message), actively gathering qualitative data (eg, surveys, focus groups, sensing sessions) from concerned populations to assist in more effective communication throughout an entire project. The HRCP also uses audience feedback tools (eg, focus groups) to pretest and validate risk communication message effectiveness, for example, examining if the information presented is understandable, and are there words and/or phrases that resonate poorly with the target audience. The HRCP can assist risk communication messengers, often scientific subject matter experts who rely primarily on quantitative data, in becoming more effective. To this end, it provides several risk communication training options: introductory, advanced, and specialized. Over the past decade, the HRCP has provided several tailored and focused training sessions to Army health physicists, the most recent being a 2-day workshop based upon an actual case study involving the potential overexposure of a Soldier to 200 cSv. (It was determined that the Soldier's dosimeter had been intentionally irradiated after the individual had worn it and turned it in.) The workshop included role-playing risk communication exercises involving actors playing the roles of the potentially exposed Soldier, his wife, and a news reporter.[7] Finally, the HRCP provides complete support to public health crisis events, including the development and implementation of a comprehensive communication strategy, identifying and engaging key audiences, and providing on-the-ground support throughout the risk communication intervention to the evaluation phase.

The following case studies fully illustrate the comprehensive support provided by the Army's health risk communication assets.

THREE MILITARY RADIATION RISK COMMUNICATION SUCCESS STORIES

The first radiation risk communication success story we present occurred in 2003, during the early phases of Operation Iraqi Freedom. High level concerns were raised about the safety of US troops occupying the Tuwaitha Nuclear Research Center, the crown jewel in Saddam Hussein's nuclear weapons program, located just outside of Baghdad. At the time, over 4,000 Soldiers and Marines were in and around the facility which had been recently bombed during coalition operations, and vandalized and looted by local Iraqis. The decision was quickly made to assemble a special scientific team from within USACHPPM and expedite its dispatch to Iraq in order to perform a thorough field assessment and communicate the risks to the US forces deployed there.[8]

Since it was obvious that this was a radiation risk communication intervention, a matrixed team combining health physics (HP) and RC expertise was formed to develop a response strategy. First, the deploying team leader was provided refresher RC training and, based on demographic information and communication preferences of the units on the ground, key RC messages for the response were developed: (*a*) the team was deployed because of Army leadership concerns about protecting their troops; (*b*) the team's mission was to ensure the safety of US forces; and (*c*) the team was comprised of the Army's foremost radiation experts. Upon arrival, the team leader immediately met with the deployed Soldiers to present the situation and explain the safety of ambient radiation levels. Once environmental samples were analyzed and the risk assessment was completed, it was determined that the Soldiers were safe (the highest upper bound dose equivalent was estimated to be 1.2 cSv, which is less than one fourth on the annual allowable dose for radiation).[9] Fact sheets were then developed and provided to the units and their direct leadership.

COL Melanson, the leader of the assessment and radiation risk communication team, describes the situation and explains the safety of ambient radiation levels to deployed Soldiers at the Tuwaitha Nuclear Research Center outside of Baghdad, Iraq in 2003. Photo provided by COL Melanson.

Complementary RC was also provided to key stakeholders at all higher echelons of command. As a direct result of this successful intervention, Soldier concerns were satisfactorily addressed and the situation never escalated to become a public affairs issue or result in congressional interest.

The second intervention occurred in early 2004, when members of the 442nd Military Police (MP) Company, New York Army National Guard, redeployed from Iraq and were inappropriately denied routine postdeployment bioassay screening for depleted uranium. Disgruntled about their lack of medical testing, some of the Soldiers approached a local paper, the *New York Daily News*, for assistance. Despite the ethical implications of becoming part of the story, the paper coordinated and funded the collection and analysis of urine bioassay samples from the Soldiers.[10] The *New York Daily News* sent the samples for analysis to the Uranium Medical Research Centre (UMRC)(Toronto, Canada), a self-proclaimed independent, nonprofit organization and alleged activist group opposed to use of depleted uranium. When the URMC sent the medical specimens to a nonaccredited geology laboratory, depleted uranium was detected (though no amounts reported) and the story immediately became headline news internationally and evoked widespread concern, including congressional inquiries.[11,12]

As in the previous case study, a matrixed team was quickly assembled with HP and RC expertise (a physician was also added to the team). The team immediately went to Fort Dix, New Jersey on a fact-finding mission to meet with and listen to Soldiers and their Families, a key first step in effectively identifying true concerns and communication needs. An environmental sampling team was also sent to the 442nd MP Company's base camp in Iraq to survey for depleted uranium (none was detected). Risk communication training was provided to the medical staff at the Fort Dix hospital and the 442nd Soldiers were finally offered bioassay testing. Even though not medically required, offering the option to be tested reinforced the critical risk communication message that the Army truly cared about Soldier welfare. Despite the offer, only about one fourth of the roughly 200 Soldiers in the unit wanted to be tested (all of the results were consistent with natural uranium and within normal levels, as reported by the Centers for Disease Control and Prevention (CDC)).[13] Once available, bioassay results were discussed with the individual Soldier, military Families, and healthcare providers. Briefings were also provided to senior National Guard leadership and select members of Congress from New York. Once again, due to the prompt and effective response, all stakeholder concerns were addressed and the crisis was successfully resolved.

The final case study event occurred in the summer of 2007, when a medic from the Army's 101st Airborne Division redeployed from Iraq. Having been to the Tuwaitha Nuclear Research Center (TNRC) during his deployment, the medic contacted the CDC to ask about the health risks of radiation exposure because of health problems he was experiencing. Fortunately, an Army physician, who was coincidentally doing a fellowship at CDC, was contacted and the matter was properly referred to the Army Medical Department. As with the 442nd MP Company, the media also became involved, though much later in the response than the previous case.

As before, a matrixed team of HP, RC and medical experts was assembled. A comprehensive RC strategy was developed to respond to all stakeholders. Support from senior Army leaders ensured full cooperation by all Army participants. Despite some initial reluctance to engage other recently redeployed Soldiers at Fort Campbell (home of the 101st Airborne), the natural inclination to "just let sleeping dogs lie" was overcome.[14] Updated information about Tuwaitha was obtained from "boots on the ground" Army HP assets to augment what was already known about the site and provided to unit Soldiers. After interviewing the medic, RC messages were developed: (*a*) the TNRC was safe; (*b*) all the radioactive sources at TNRC had been properly contained and safely stored; (*c*) anyone desiring testing could provide a bioassay sample. Given that, preparations were made for the "nightmare scenario" of hundreds of individuals simultaneously wanting bioassays. As it turned out, however, only the medic ultimately wanted to be tested (his results were either below detection limits for anthropogenic radioactive sources located on the site or within

CDC reported dietary levels for other naturally occurring radionuclide).[15] In order to assist other potentially concerned Soldiers, a combined HP and RC team deployed to Fort Campbell (where an onsite medical expert joined the team) and 3 town hall meetings were held for Soldiers, their Families, and members of the local press. By delivering the actual briefing prepared for the Soldiers to the assembled reporters, the team leader was able to tell the good news story that the Army was genuinely concerned and was making sure that its Soldiers were safe. During all of the town hall meetings, the team's RC expert observed the HP's message delivery and response to questions, and provided real-time feedback to enhance the process. Once again, the concerns of stakeholders were addressed and the crisis was satisfactorily resolved. Feedback from the initially concerned medic and his fellow Soldiers indicated the response was effective, and press reports were very favorable overall.[16]

SUMMARY AND CONCLUSIONS

Risk communication is more than just a message; it is both a discipline and a process. Military radiation risk communication shares all of the difficulties of communicating civilian radiation risks along with its own unique challenges. Effective risk communicators address all three of the components of communication: audience, messenger, and message, specifically in that order. The Health Risk Communication Program is a vital corporate asset of the United States Army that provides unique and essential expertise to enhance risk communication, whatever the risk. The proper partnering of health physics and health risk communication expertise, coupled with senior leadership support, allayed public concerns and diffused 3 high stakes crises, despite media involvement and Congressional scrutiny in two of them. As illustrated in the case studies discussed, effective risk communication is actually achievable and we firmly believe that without it, properly responding to crises, actual or perceived, is impossible.

RECOMMENDATIONS

Recognizing that risk communication is a discipline and a process, not merely a product, is essential for success. All health physicists should add risk communication training as part of their professional development, and integrate risk communication into their ongoing professional practice, and not just during emergencies. Whenever possible, health physicists should seek to partner with competent health risk communicators in a matrixed team, thereby exploiting the synergy between these 2 diverse, yet complimentary disciplines. Finally, health physicists should also share their risk communication success stories, along with their failures, so others can learn from their experiences.

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Summary of the Infectious Diseases and Disaster Response Conference in Abu Dhabi

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Due to the interconnectedness of the world today and the ease with which infectious diseases can spread globally, collaboration within and among countries around the world on pandemic planning and response is immensely important. One of the first steps for pandemic planning involves identifying existing gaps in a nation's current plans, and examining previous outbreaks for lessons learned. To identify such gaps, the World Health Organization (WHO) created a framework with 5 main components for assessing disaster and pandemic planning and response: surveillance, healthcare response, public health intervention, communication, and command.[1]

Assessing a country's current pandemic planning and response capability can be accomplished by examining each of the 5 aspects of the WHO framework.[1] The first component is surveillance. Countries need a robust surveillance system to detect emerging infectious diseases or potential outbreaks. However, many countries may lack the capacity for such a system. In resource-limited settings or countries recovering from a disaster, foreign militaries can play a key role in disease surveillance.[2] Foreign militaries may have the capacity to support local civilian ministries of health in disease surveillance and reporting. Healthcare response focuses on ensuring that current hospital resources meet demands and that contingency plans exist for continuity of operations during an emergency. Public health intervention is important for preventing or containing the spread of disease. Strategic communication is also a fundamental part of disaster response and should occur pre-event, during the event, and during response and recovery phases. Finally, the incident command structure is an integral aspect of disaster response. Identifying roles and responsibilities of key staff and cross-training ahead of time will enable individuals to better respond in the event of a disaster.

The WHO framework provides a structure to assess a country's pandemic response capabilities, but many countries around the world may lack capacity for efficient and effective pandemic response. The Armed Forces Health Surveillance Center–Global Emerging Infections Surveillance and Response System (AFHSC-GEIS) partners with many countries around the world to build sustainable public health surveillance and laboratory capacities.[3] In addition to partnering with laboratories around the world, the AFHSC, in collaboration with the Center for Disaster and Humanitarian Assistance Medicine* (CDHAM) and the geographic combatant commands, also works closely with partner nation militaries and local ministries to conduct training workshops and exercises on important topics such as infectious diseases and disaster response.

THE CONFERENCE

The AFHSC, collaborating with the CDHAM and the US Central Command, held the Infectious Diseases and Disaster Response Conference (IDDRC) July 11-14, 2011, hosted by the United Arab Emirates Armed Forces in Abu Dhabi. This conference promoted regional interoperability and enhanced the capability of regional countries to respond to complex humanitarian and health emergencies, with a particular focus on response to infectious disease-related disasters such as pandemics.

Specific objectives of the conference included:

a. Creating a platform for military leaders and civil authorities to share best practices and lessons learned in regards to emerging infectious diseases and disaster response;

b. Providing regional partners with current updates on global emerging infectious threats and surveillance improvement;

c. Assisting regional partners to understand the roles of the international community (international organizations, nongovernmental organizations, and regional governments) in the management of disasters; and

*The Center for Disaster and Humanitarian Assistance Medicine is a component of the Department of Military and Emergency Medicine at the Uniformed Services University of the Health Sciences in Bethesda, Maryland. The Center is the Department of Defense's focal point for academic aspects of medical stability operations.

d. Identifying national and regional opportunities for improvement of surveillance and regional coordination that will assist in the development of a regional response to emerging infectious hazards and disasters.

The conference included a total of approximately 95 distinguished participants and lecturers from the United Arab Emirates, the United States, Iraq, the Hashemite Kingdom of Jordan, Lebanon, Qatar, the Kingdom of Saudi Arabia, and Yemen. Individuals from each of the countries were invited to present on best practices in disaster mitigation within their countries. Subject matter experts from the US Department of Homeland Security, AFHSC, Georgetown University, the George Washington University, and the Carolinas Medical Center presented on topics relevant to infectious disease and disaster response. The conference incorporated facilitated discussions on the Sphere Project,[a] strategic communication in disaster response, and the World Health Organization's International Health Regulations.[4] Attendees had the opportunity to participate in a regional pandemic response tabletop exercise, which identified gaps and promoted regional communication.

During the conference workshops, participants were able to identify existing gaps in their current national and regional plans and methodologies, examine previous infectious disease outbreaks for lessons learned, consider possible ways to address some of the identified gaps, and build relationships with their regional counterparts. Future interactions will fortify regional partnerships and cooperative agreements to strengthen infectious disease and disaster response within the region.

Emerging Infectious Diseases and Surveillance

The Institute of Medicine[b] defines emerging infectious diseases as conditions that have increased incidence in humans and that are clinically distinct.[5] In his lecture "Emerging Infectious Disease Updates," Dr Daniel Lucey[c] presented information on newly emerging diseases as well as disease prevention strategies for controlling outbreaks. He further distinguished between emerging and reemerging infectious diseases, explaining that emerging infectious diseases are newly recognized pathogens, such as Nipah virus, Severe Acute Respiratory Syndrome (SARS), and pandemic (H1N1) 2009. Reemerging infectious diseases have been previously recognized, but are now seen in a new location, such as Monkeypox, West Nile virus, and Rift Valley fever.

Dr Lucey was an integral part of a response team to the 2003 SARS epidemic in Toronto, Canada. He elaborated upon several measures for preventing disease transmission, including the importance of SARS assessment triage centers that were established in trailers outside of hospitals to prevent the spread of disease while still providing care for sick patients. Having dedicated staff members to deal with SARS patients, screening check points at all hospital entrances, and providing adequate personal protective equipment for patients provided further mechanisms for reducing transmission within the hospital.

Dr Lucey outlined 5 major contributions of the early 21st century regarding emerging infectious diseases: (1) the One Health concept (collaboration of human, animal, and environmental health disciplines, see http://www.onehealthinitiative.com/index.php); (2) the importance of international partnerships; (3) antibiotic and antiviral drug resistance; (4) the WHO International Health Regulations[4] (IHR), and (5) global infectious disease surveillance. Dr Lucey also mentioned the importance of organizations such as the AFHSC-GEIS and the WHO in current global surveillance efforts.

The AFHSC-GEIS laboratory surveillance network has been an important player in global biosurveillance of infectious diseases. In 2009, AFHSC-GEIS provided funding and oversight to a network of 39 partners at approximately 500 sites, impacting a total of 92 countries through active surveillance projects, capacity-building initiatives, or participation in training exercises.[6] Many of these training initiatives have been in direct support of the WHO IHR.[7] During the IDDRC, COL Robert Lipnick[d] and Ms Priya Baliga[e] led a small-group session on the importance of the WHO IHR, and reviewed the framework for a member state to report a potential Public Health Emergency of International Concern (PHEIC). They also introduced scenarios of potential PHEICs and

[a] The Sphere Project, headquartered in Geneva, Switzerland, is a voluntary initiative that brings a wide range of humanitarian agencies together to improve the quality of humanitarian assistance and the accountability of humanitarian actors to their constituents, donors, and affected populations. Information available at: http://www.sphereproject.org/.

[b] The Institute of Medicine is the health component of the National Academy of Sciences. It is an independent, nonprofit organization that works outside of government to provide unbiased and authoritative advice to decision makers and the public. Information available at: http://www.iom.edu/About-IOM.aspx.

[c] Adjunct Professor, Department of Microbiology and Immunology, Georgetown University

[d] Director, Communications Standards and Training Divisions, AFHSC

[e] Epidemiologist, AFHSC

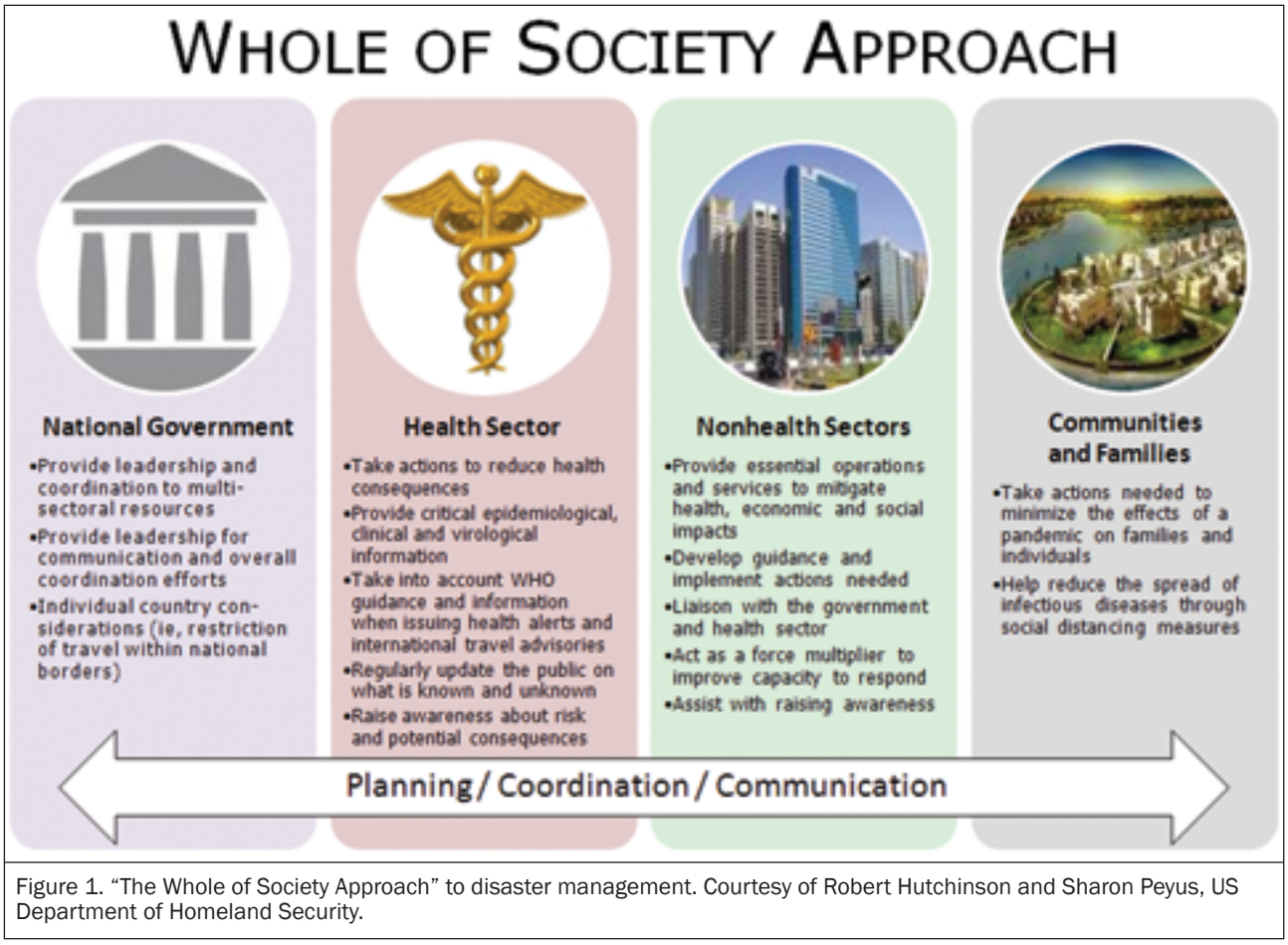

Figure 1. "The Whole of Society Approach" to disaster management. Courtesy of Robert Hutchinson and Sharon Peyus, US Department of Homeland Security.

led discussions on the intricacies of reporting through a country's ministry of health to the WHO. Further discussions deliberated upon the consequences of countries not disclosing a potential PHEIC to the WHO, the criteria that constitute a PHEIC and when to report, and complications of detecting and reporting potential PHEICs in countries with limited resources and capacities. Thus, infectious disease surveillance is only the first step to overall disaster planning and response.

Disaster Planning and Response

When planning for disasters such as an infectious disease outbreak or pandemic situation, it is extremely beneficial to have a whole of society approach to the disaster, including integrated planning and preparation (Figure 1). Mr Robert Hutchinson[a] and Ms Sharon Peyus[b] highlighted the importance of identification of essential sectors in supporting relevant authorities, and the need for development of business continuity plans for critical sectors. They also emphasized the benefits of multisectoral preparedness through close interministerial collaboration and communication, commitment of subject matter experts, and the leveraging of resources.

In their lecture on contingency planning, they presented a variety of lessons learned during the US response efforts for Hurricane Katrina in 2005, the H1N1 pandemic in 2009, and the earthquake in Haiti in 2010. The after action report (AAR) on the H1N1 pandemic evaluated several functional areas, including emergency operations center management, information gathering and recognition of indicators and warnings, responder safety and health, critical resources logistics and release, and managing risk. Some of the strengths of the pandemic (H1N1) 2009 response, including previously conducted H5N1 planning and training efforts, greatly assisted the development and refinement of pandemic mitigation measures. Assessment of the AAR established that DHS effectively communicated with state, local, territorial, and tribal government officials. Some of the areas of improvement noted in the AAR included increasing efficiency of incident command/control to streamline

[a] Assistant Special Agent in Charge, US Department of Homeland Security
[b] Director, National Incidence Response Unit, US Department of Homeland Security

decision making, improving communication, information sharing, and strengthening partnerships.[8]

The US Federal Emergency Management Agency's Incident Command System (ICS)[9] is a standardized, on-scene, all-hazards incident management approach. In his lecture "Force Health Protection in Disaster Response," Dr David Callaway* discussed the ICS, which helps to manage incidents through concepts such as unity of command, common terminology, management by objective, flexible and modular organization, and span-of-control (Figure 2). The ICS has an systematic approach to managing safety of responders through 4 functions of the safety management cycle: information acquisition, analysis of options, decision-making, and taking action. He emphasized force health protection as an essential element for maintaining resources to respond to crises through standardized processes and customized response, and that there is a full spectrum of requirements throughout the disaster and deployment cycle.

In addition to ICS and the importance of force health protection, Dr Callaway also spearheaded a small group discussion on strategic communication during a disaster, deemed critical during all stages of the event. He provided 2 case examples: Hurricane Katrina and the consequences of failed strategic communication, and the success of strategic communication during the earthquake in Haiti. During the Katrina event, the lack of a basic coordinating instruction, a knowledge management plan, and an overall situational awareness was the reason that strategic communication failed. In contrast, successes in Haiti were attributed to a clear strategic message, which allowed decentralized command and execution. Additionally, relationships with the press and nongovernmental organizations (NGOs) were firmly established prior to the earthquake. These relationships fostered a sense of trust between all parties involved and were offered as further reasons why strategic communication was so effective (Figure 3).

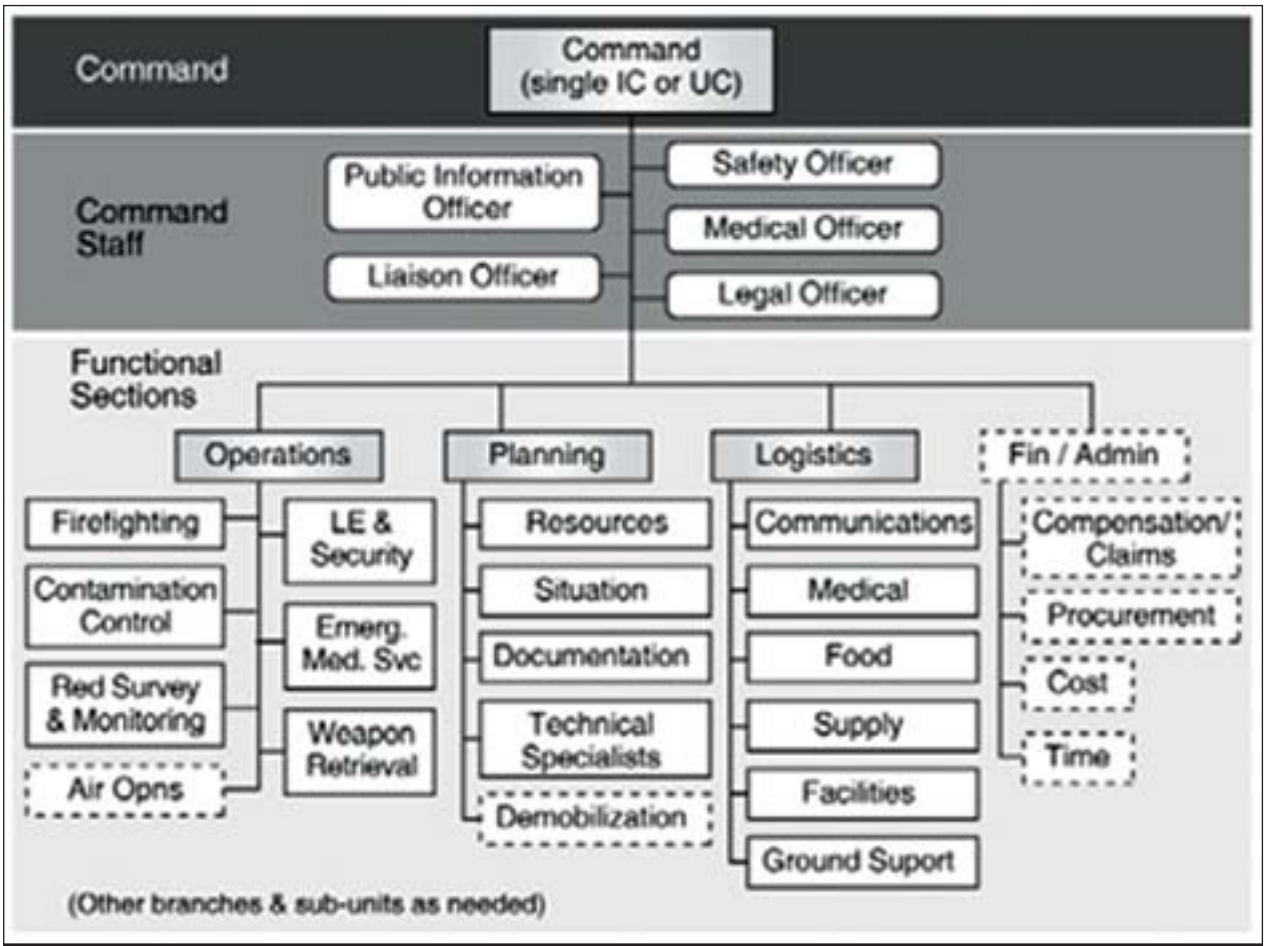

Figure 2. The structure of a nominal Incident Command System (US Dept of Homeland Security).[9]

*Director, The Operational Medicine Institute and Assistant Professor of Emergency Medicine, Carolinas Medical Center, Charlotte, NC

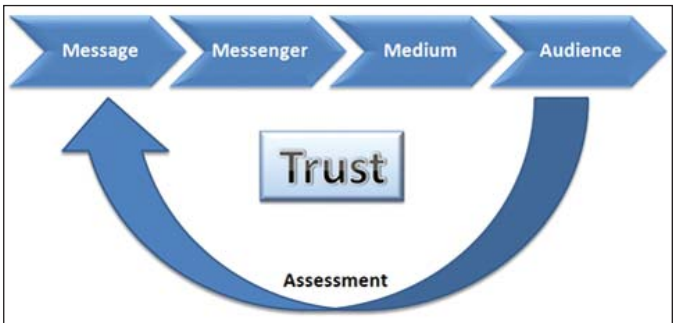

Figure 3. Five elements of communicating a message. Source: Dr David Callaway.

Nongovernmental organizations play a key role in response to disasters. During the IDDRC, Dr David Hajjar* guided a small-group discussion on the Sphere Project and the Sphere Handbook,[10] which is designed for use in disaster response and is applicable to natural disasters as well as armed conflict. During the small-group discussion, Dr Hajjar presented a case study on a migration of Ugandans crossing the border to Tanzania after a pandemic outbreak resulted in violence and limited resources.

Throughout the session, discussions arose about minimum and adequate standards for conditions in refugee camps, when and how refugees should be persuaded to return to their countries of origin, and various other factors that must be taken into account when caring for individuals fleeing a crisis (security, political situation, weather, health, capacity, mental state, etc). A lively debate surrounded the topic of adequate standards for camps without indulging refugees in luxuries, so they would still feel compelled to return to their countries of origin once the emergency has been resolved. The importance of maintaining dignity while balancing the desire to return home was recognized as a pervasive challenge in the refugee camp setting.

Best Practices in Disaster Response: Country Presentations

The IDDRC was designed to foster contribution by attendees. Participants from invited countries were encouraged to make presentations on best practices in disaster response within each of their respective countries. Dr Saleh Fares, a consultant for the Emergency Department at the Zayed Military Hospital in Abu Dhabi, presented "Regional Critical Infrastructure and Key Resources for Disaster Response" within the United Arab Emirates (UAE). The unique population of the UAE, which is made up of more than 80% guest workers with thousands of people crossing borders daily, makes responding to disasters in the country a particular challenge. One of the key resources Dr Fares highlighted for managing disasters in UAE was the Higher National Security Council National Emergency and Crisis Management Authority. He discussed the command structure, starting with Level 1–the President of the UAE; Level 2–the National Security Advisor; Level 3–the Ministries of Health, Interior, and Environment; and Level 4–local or Emirate level. He shared the UAE's national response plan to H1N1,[11] including surveillance elements, case investigation and management, strengths, and areas for improvement. Strengths of the H1N1 response included adequate funding, political support, coordination of various organizations, and incorporation of past lessons learned. Areas for improvement included lack of a "real-time" surveillance system, limited laboratory capacity and capability, poor communication, and suboptimal ICS coordination.

Dr Mahmud Abdallat, representing the Preventive Medicine Department of the Royal Medical Service (RMS) of Jordan, lectured on Jordan's pandemic influenza response. He explained that the RMS had representatives on the National Steering Committee, National Technical Committee, and National Treatment Committee for Pandemic Influenza Response, and that the RMS pandemic response plan is part of the national response plan for pandemic influenza in Jordan. This response plan encompasses the RMS surveillance system, external communications, and their internal communications, including RMS medical outpatient clinics and military units. These communication systems are coordinated by a senior officer in a designated operations room in the RMS directorate. Dr Abdallat also described the structure of teams for surveillance and treatment of pandemic influenza cases in RMS hospitals.

Dr Awni Abulail, also of the Jordanian RMS, gave a briefing on the RMS role in international medical assistance. Dr Abulail presented some of the capabilities of the main RMS hospital, King Hussein Medical Center, (http://www.jrms.gov.jo/Default.aspx?tabid=54), as well as Jordan's field hospitals and surgical teams and facilities, who have provided medical assistance to 21 locations throughout the world as part of United Nations Peacekeeping Forces or humanitarian aid missions.

Brigadier General Maurice Sleem, Surgeon General of the Lebanese Army, addressed the nature of disasters in Lebanon, most of which are man-made and involving wars. He reviewed response efforts to a major oil spill in the Mediterranean Sea as a result of the bombing of the Jiyeh power station, which caused the leakage of nearly 15,000 tons of oil reaching areas on the Syrian coastline, and the waters of Turkey and Cyprus. The Lebanon

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Ministry of Environment requested assistance from 31 countries, 71 national and international organizations, and 80 NGOs in response to this disaster. The quantity of waste collected and safely stored is estimated to be about 200 cubic meters, however, there are still 12 polluted sites undergoing cleanup operations along the shoreline of Lebanon.

Brigadier General Sleem discussed health security in relation to the response to unexploded ordnance in Lebanon where the Ministry of Public Health continues to provide emergency medicine and supplies for acute and chronic conditions. He shared the role of the WHO and the United Nations Children's Fund in health security in support of response efforts. These organizations worked with a broad range of partners in Lebanon to save lives, protect civilians, and support basic services such as health, water, sanitation, education, and psychosocial care.

Brigadier General Sleem added that Lebanon's National Committee for Disaster Management held its first national conference on disaster management in November 2009, bringing together many key stakeholders. However, due to current political circumstances in Lebanon, there has been a delay in setting plans and policies, and in strengthening infrastructure development in the country.

Major General Dr Samir Abdullah Hasan from the Directorate of Iraqi Military Medical Services gave a presentation on the "Emergency Health Plan of the Iraqi Surgeon General's Office." The Iraqi plan's purpose is to establish a policy under the new democratic Iraqi law to protect military and civilian installations, facilities, and personnel in the event of a public health emergency, whether due to manmade or natural disaster, outbreaks of infectious disease, biological warfare, or terrorism. The plan contains elements of isolation, public health emergency response, quarantinable communicable diseases, and mandatory quarantine. It also addresses those public health emergencies that occur during religious events.

The Iraq Surgeon General's office has a senior military medical office or Ministry of Defense civilian employee who is designated as a Public Health Emergency Officer (PHEO). The PHEO ascertains the existence of cases suggesting a public health emergency; investigates cases for sources of infection; recommends implementation of proper control measures; defines distribution of illness; identifies all exposed individuals; counsels individuals on the course and spread of their illness; assesses facilities for purpose of closing; evacuates affected individuals; decontaminates or destroys any materials contributing to the public health emergency; shares information with federal, provincial, or local officials; notifies applicable military channels; and reports public health emergencies to the Surgeon General and the Ministry of Defense. The PHEO is also responsible for providing written notice to all quarantined individuals, screening and safely disposing of corpses, and informing all affected individuals of control and mitigation actions to take during a public health emergency. Dr Samir listed education and training of PHEOs and commanders as one of the most pressing challenges faced by the Iraq Surgeon General's office for emergency health planning.

The country presentations were an essential element of the IDDRC. Through learning about country-specific approaches to controlling infectious diseases and responding to disasters, groups were better able to interact and have open discussions during the tabletop exercise (TTX) portion of the conference.

Regional Pandemic Tabletop Exercise

The UAE Regional Pandemic TTX was based on an outbreak of influenza-like illness that initially presented in a rural village in Thailand. The participants were divided, as much as possible, into 4 groups with representatives from all of the countries present. Each group was given about 3 hours to discuss the exercise before reconvening to present the main findings of their discussion. Participants were encouraged to take the information gained from the previous days' lectures to guide their TTX group discussions.

The objectives of the TTX were to:

- Create a platform for military leaders and civil authorities to share best practices and lessons learned in emerging infectious disease and disaster response activities.
- Provide regional partners with current updates on global emerging infectious threats and surveillance.
- Assist regional partners in understanding the roles of the international community (international organizations, NGOs, regional governmental organizations) in the management of disasters.
- Promote regional interoperability and enhance the capability of host nations to respond to complex humanitarian emergencies.
- Identify national and regional opportunities for the improvement of surveillance and regional coordination that will assist in the development of multicountry response to emerging infectious hazards and disaster response.

Sharing best practices and lessons learned throughout the region, each country contributed specific experiences and significant discussion points to the exercise. In one group, representatives from both the UAE and Jordan noted having a national food stockpile for emergency purposes, which can feed their respective country's current populations for 3 to 6 months. As part of their national pandemic response plan, the Jordanian government has informed retired medical personnel who, in case of a pandemic, may be recalled to service. The Jordanian plan has the capability to increase personnel capacity by 30% through extending normal emergency department shifts from 8 to 12 hours.

Timely, accurate and effective communication is critically important during disasters as it contributes to saving lives and increases the public awareness and understanding. Discussions from the breakout groups focused heavily on effective communication practices during the pandemic scenario. Participants from the UAE and Jordan discussed how they would strategically choose one government representative as a spokesperson, who would serve as the only source of communication between the government and the media. This trusted individual would be responsible for relaying facts, dispelling rumors, calming the public, and delivering the government's messages about the pandemic. This open communication with the media would help inform and empower, building trust among the government, the media, and the public.

Another critical issue for pandemic response planning is that of refugees and internally displaced persons (IDPs). Concerns arose, such as prioritizing resources between refugees and citizens, focusing on high risk individuals within these groups, as well as exploring legal mandates on basic needs of refugees, communicating with the refugees' country of origin, and enforcing border security. One Jordanian participant noted that during a severe crisis, schools would be closed and therefore could be used as a shelter to house IDPs, whereas refugees would be in camps and the burden would remain on the refugee field hospitals to treat those sick individuals. It was concluded that it is essential to include neighboring countries in pandemic planning efforts.

Groups identified 2 major gaps during their TTX discussions. First, they found the countries' postpandemic planning insufficient. The transition to long-term recovery and the resources needed for the postpandemic phase was unclear. Additionally, they found that the role of NGOs and the UN during a pandemic in the region was vague. The groups additionally alluded to the relevance of the One Health concept, emphasizing the importance of human and animal disease surveillance and the integration of human and animal health in the control of pandemics.

One of the facilitators noted that most of the participants in his group consisted of high-level officials who have not previously focused on contingency plans for some of their critical resources. For example, contingency plans in many countries did not appear to address employee absenteeism, a phenomena that could greatly affect the size of the critical workforce during a pandemic situation. A concrete plan did not seem to be in place in many of the participating countries for securing borders and supporting the interior with the limited personnel and resources that would be expected in such a crisis. Some countries may not have previously planned for the ramifications of interrupted electricity, sanitation pickup, food deliveries, medical supply deliveries, etc. Although most of these examples represent worst-case scenarios, it is important for countries in the region to have these components incorporated into their contingency plans for pandemics and disasters.

CONCLUSIONS

Over the course of the 4-day conference, a number of common themes were brought to the forefront by the presentations, discussions, and tabletop exercise. Among these is the need to be proactive by taking steps to identify, detect and respond effectively to crises rather than wait for a disaster to occur and then be dependent on external organizations (such as the UN) to address national needs. Participating national governments understood their role as the primary responsible party for the wellbeing of their citizens. The attendees understood the value of being proactive, taking immediate action, and enhancing their own capacity to prepare for, respond to, and recover from emergency and disaster situations. Participants expressed their appreciation for the value of the multisectoral, all-hazards, whole of society approach and pronounced their desire to move forward with such a comprehensive plan.

In addition, participants exhibited a new appreciation for (1) the value of redundancy in emergency management procedures and methodologies; (2) the importance of continuity of operations and continuity of governance procedures; and (3) the critical role of effective coordination and information-sharing mechanisms for enhancing timeliness and effectiveness of their disaster response efforts. Finally, due to the wealth of participant experience and breadth of geographical representation, participants embraced the importance of developing clearly articulated and tested standard operating procedures, coupled with written mutual aid agreements and

procedures at the local, national, regional, and international levels.

As a result of the time shared together at the workshop, participants identified existing gaps in their current national and regional plans and methodologies, examined previous infectious disease outbreaks for lessons learned, considered possible ways to address some of the identified gaps, and built relationships with their regional counterparts.

The IDDRC was one of several workshops planned by the AFHSC, the Center for Disaster and Humanitarian Assistance Medicine, and US combatant commands that focus on emerging infectious disease outbreaks and disaster planning and response as a means of contributing to national, regional and global security.[12] These engagements (workshops, conferences, tabletop exercises) bring together civil and military personnel to develop common strategies on surveillance, laboratory techniques, implementation of public health policies, use of vaccines, and military support for an effective pandemic response. The information obtained from this conference in Abu Dhabi will help to inform ongoing efforts to improve capacity while enhancing the capability of regional countries to respond to complex humanitarian and health emergencies.

Preparing globally to address pandemics is a significant challenge, but the consequences of being unprepared could be catastrophic. Meeting this challenge will require a comprehensive, multidisciplinary approach to building sustainable capacity in partner nations to recognize, prevent, and respond to the threat of emerging and reemerging infectious diseases which are critically important for an effective global response. Additionally, future engagement strategies will be designed to focus on regional partnerships and cooperative agreements geared towards strengthening infectious disease and disaster response efforts.

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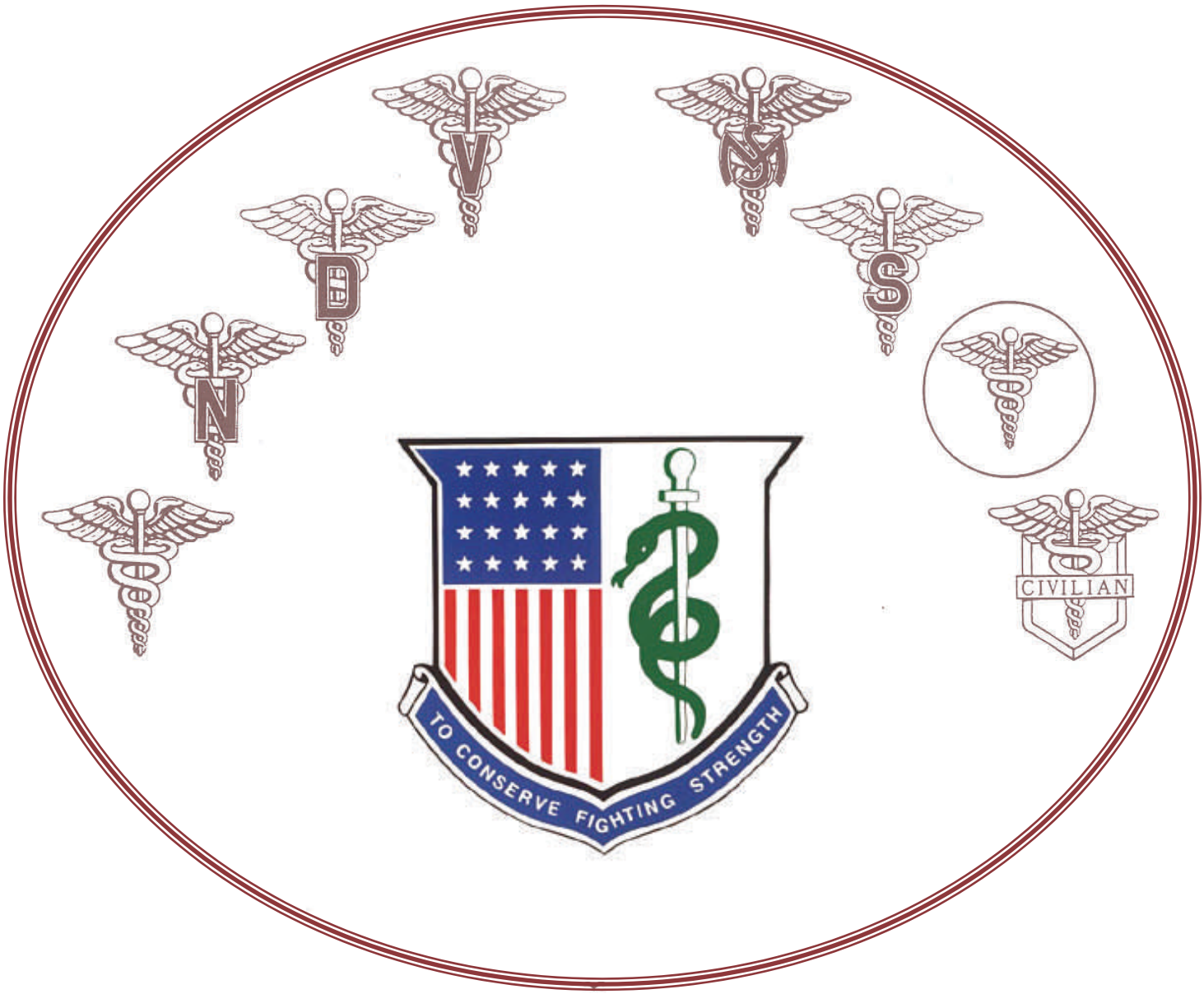

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